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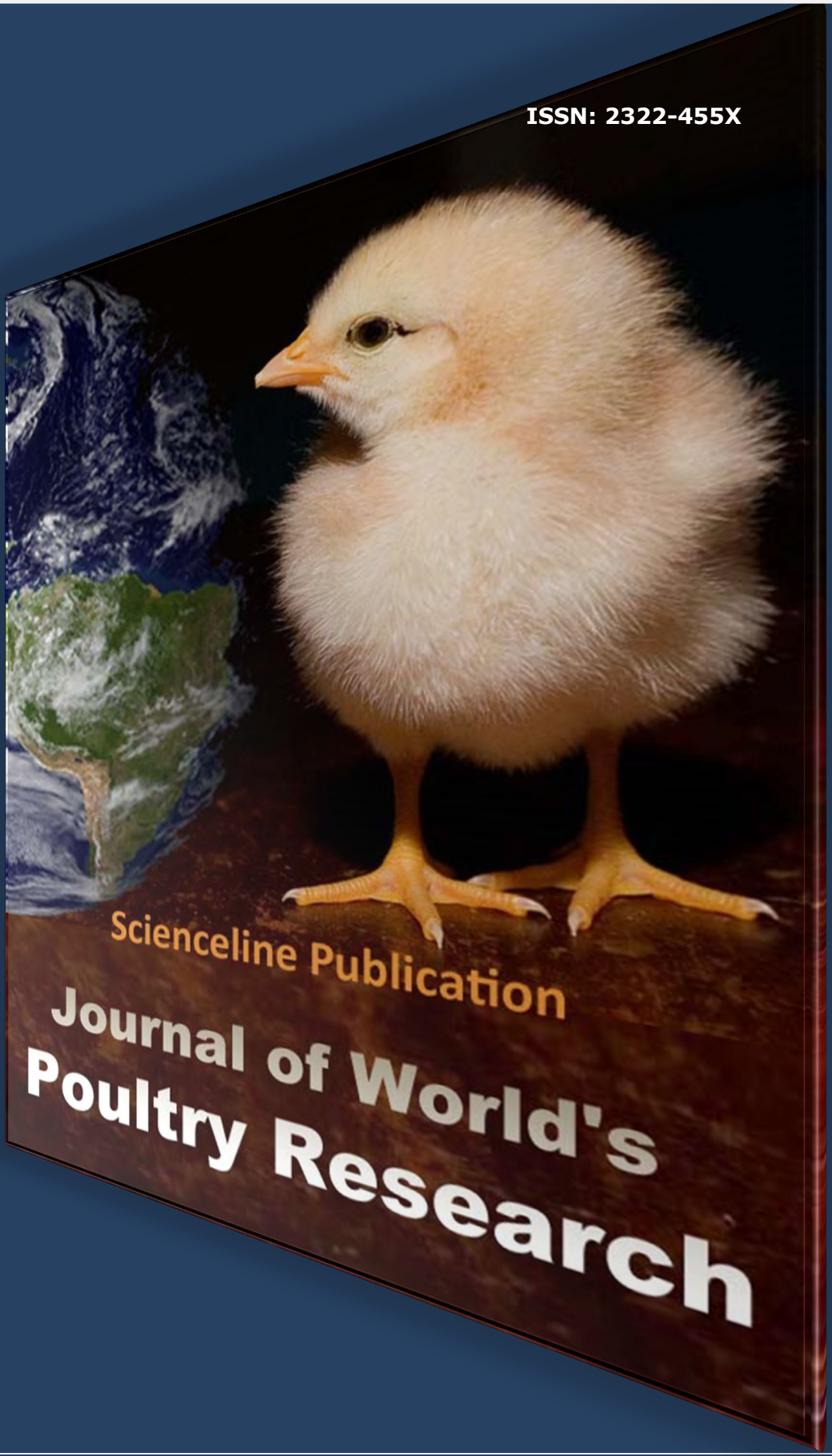

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Research Paper

Effects of a Multi-Genus Synbiotic (PoultryStar® sol) on Gut Health and Performance of Broiler Breeders

Prentza Z, Castellone F, Legnardi M, Antlinger B, Segura-Wang M, Kefalas G, Fortomaris P, Papaioannou AAN, Stylianaki I, Franzo G, Cecchinato M, Papatsiros V, and Koutoulis K.

J. World Poult. Res. 12(4): 212-229, 2022; pii: S2322455X2200024-12
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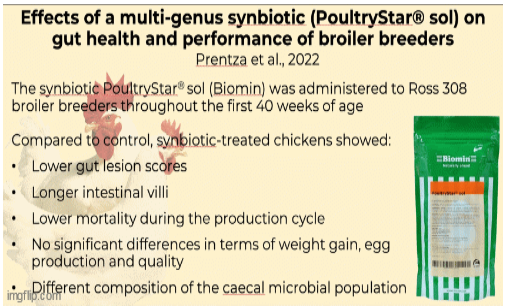


ABSTRACT: In recent years, a rising interest has been directed towards the use of nutraceuticals in the zootechnical sector, including probiotics, prebiotics, and synbiotics, as a way to support production efficiency and cope with the increasing limitations to the use of antibiotics. In poultry, however, most studies on these products have been conducted on broilers, while less information is available on their benefits to other productive categories. The present field study aimed to assess the effects of a multi-species synbiotic product (PoultryStar® sol) on the gut health and productive performance of broiler breeders. A total of 24761 day-old Ross 308 parent stock chicks were acquired from a single hatchery and placed on the same farm. Female chicks were divided into three groups and raised in different houses (A, B, and C), in which males were introduced at the age of mating and followed until 40 weeks of age. The synbiotic was provided by drinking water to the flocks in houses A and B, while house C was kept as control. Following the manufacturer's guidelines, the product was administered intermittently once every two weeks, except in the first and the twenty-first week when it was supplied for three consecutive days. Data on performance parameters, egg quality traits, bacterial enteritis scoring, intestinal morphometry, and histopathology were recorded, and the caecal content was collected at 15, 25, and 40 weeks of age to investigate the intestinal microbiota using high-throughput next-generation sequencing. Synbiotic-treated hens showed significantly higher survivability during production compared to the control group. No clear differences were observed between treated and control chickens in terms of egg production and quality, and the effect of the synbiotic on weight gain also appeared limited. From 25 weeks onwards, synbiotic-treated chickens scored better in terms of macroscopical lesions and had longer intestinal villi. Significant differences in crypt length and histopathological lesions were also found at multiple sampling points. A treatment effect on caecal bacterial composition was detected with a differential abundance of Gastranaerophilales, Lachnospiraceae, Helicobacter, Ruminococcaceae, and Clostridia, among others. Taken together, obtained results support the beneficial effects of the intermittent administration of the synbiotic product PoultryStar® sol on the gut health of broiler breeders.
Keywords: Broiler breeder, Gastrointestinal health, Histopathology, Microbiota, Synbiotic

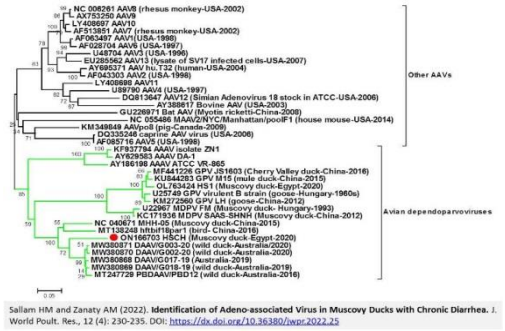
[Full text-PDF]

Research Paper

Identification of Adeno-associated Virus in Muscovy Ducks with Chronic Diarrhea

Sallam HM and Zanaty AM.

J. World Poult. Res. 12(4): 230-235, 2022; pii: S2322455X2200025-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.25



ABSTRACT: Adeno-associated viruses (AAVs) are defective members of the genus Dependoparvovirus. Waterfowl parvoviruses, another member of the Dependoparvovirus, were found to be the closest relative of AAVs. This study was performed to identify the genetic changes that may occur to goose parvovirus (GPV) in one Muscovy duck flock that was observed for 12 weeks after the virus was isolated. Persistent watery diarrhea and wing deformity were the common signs. Cloacal swabs were collected from diseased ducks. Unexpectedly, the identified virus was an AAV. The closest strains were duck AAVs at the nucleotide level, identified in Australia and China. Meanwhile, only 52.3% of nucleotide identity was shared with the GPV strain, previously identified from this flock. Duck

adenovirus (DAdV) could not be identified in the samples. This study is one of the first studies in which genetic changes of GPV were tracked. In addition, emerging duck AAV from GPV is suggested, which will be useful for future virus classification.
Keywords: Adeno-associated virus, Chronic diarrhea, Muscovy ducks

[Full text-PDF]

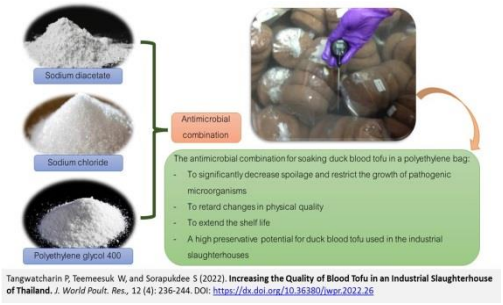
Research Paper

Increasing the Quality of Blood Tofu in an Industrial Slaughterhouse of Thailand

Tangwatcharin P, Teemeesuk W, and Sorapukdee S.

J. World Poult. Res. 12(4): 236-244, 2022; pii: S2322455X2200026-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.26

ABSTRACT: Blood tofu, or cooked duck blood curd, is a Chinese delicacy in East Asia. Its quality and shelf-life are low due to microorganism contamination during production. Therefore, the present study was performed to investigate the role of sodium diacetate (SD), sodium chloride (NaCl), and polyethylene glycol 400 (PEG) combinations in increasing the quality of blood tofu. A total of 45 cooked duck blood curd samples were randomly divided into 3 groups with 3 replicates per group. The first two groups were used to investigate the effect of SD, NaCl, and PEG combinations on microbiological and physical analyses for non-inoculated samples. Another group was used to determine the effect of antimicrobial combinations on *Lactobacillus plantarum*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus* in inoculated samples that were inoculated with these bacteria. All groups were treated with control-sterilized water, 0.15% SD (w/v) + 1.25% NaCl (w/v), 0.30% SD (w/v) + 1.25% NaCl (w/v), 0.15% SD (w/v) + 0.15% PEG (w/v), and 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). The results indicated that soaking cooked duck blood curd samples in antimicrobial agent combinations could reduce mesophile and psychrophile bacteria counts in non-inoculated samples. Additionally, 0.15% SD + 1.25% NaCl + 0.15% PEG combination had a higher reduction in mesophile and psychrophile counts, compared to soaking the samples in 0.30% SD + 1.25% NaCl, 0.15% SD + 1.25% NaCl and 0.15% SD + 0.15% PEG combinations. Similarly, this combination showed a significant decrease in lactic acid bacteria, *Pseudomonas*, *Salmonella*, *Escherichia coli,* and *Staphylococcus aureus* counts in inoculated samples. Furthermore, soaking the samples in 0.15% SD + 1.25% NaCl + 0.15% PEG combination did not negatively affect the samples' physical quality. Soaking the samples in 0.15% SD + 1.25% NaCl + 0.15% PEG combination inhibited the growth of mesophile, psychrophile, and *Pseudomonas* in non-inoculated samples after storage for 10, 6, 10, and 8 days in a slaughter warehouse at 7℃, respectively, and extended shelf-life of samples for 16 days. Regarding physical quality changes, this treatment delayed the reduction of pH, hue, hardness, and chewiness of the samples after storage for 10, 8, 12, and 10 days, respectively. Thus, SD, NaCl, and PEG combination had a high preservative potential for cooked duck blood curd used in industrial slaughterhouses.
Keywords: Blood curd, Duck, Organic acid salt, Polyethylene glycol, Quality changes



Tangwatcharin P, Teemeesuk W, and Sorapukdee S (2022). **Increasing the Quality of Blood Tofu in an Industrial Slaughterhouse of Thailand**. *J. World Poult. Res.*, 12 (4): 236-244. DOI: https://dx.doi.org/10.36380/jwpr.2022.26

[Full text-PDF]

Research Paper

Characterization and Typology of Traditional Poultry Farming Systems in Southern Niger

Moustapha A, Adamou A, and Talaki E.

J. World Poult. Res. 12(4): 245-257, 2021; pii: S2322455X2200027-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.27

ABSTRACT: An appropriate agricultural policy that integrates knowledge of endogenous poultry practices should enhance household resilience by contributing to food and nutrition security and sustainable development in developing countries. The current cross-sectional survey aimed to characterize poultry breeding systems and identify types of traditional poultry farmers in Maradi and Zinder in southern Niger. Therefore, 600 households were investigated for the socio-economic parameters of poultry farmers, the breeding methods, the zootechnical parameters of the local chicken, and the health parameters relating to biosecurity and animal care. The results of the descriptive analyses indicated that traditional poultry activity is mainly carried out by men (73.5%) and small farmers (74.2%). Breeding management was primarily free-range


Moustapha A, Adamou A, and Talaki E (2022). **Characterization and Typology of Traditional Poultry Farming Systems in Southern Niger**. *J. World Poult. Res.*, 12 (4): 245-257. DOI: https://dx.doi.org/10.36380/jwpr.2022.27

breeding (99.3%). The majority of the surveyed herders (67.8%) were illiterate. However, 41.5% of them attended traditional Islamic Koranic schools. Most farmers (80%) were small-scale livestock farmers with an average herd size of 22 ± 24.9. The poultry raised were 93.3% local breeds, with chicken domination (66%). The housing did not meet the required standards, and the feed was mainly cereals. The female chicken can potentially produce 12.64 fertile eggs per clutch and brood 3.53 times per year. The leading cause of mortality in poultry was avian diseases (93.7%) and Newcastle disease in some cases. Poultry vaccination against Newcastle disease was reported by 31.5% of respondents. Of the respondents, 20% have partially observed hygiene and biosecurity measures. About 35.5% of the participants reported the provision of veterinary care, while 44% used phytotherapy to prevent or treat poultry diseases. Based on the results of this cluster analysis, three classes of poultry farmers were distinguished, each with specific characteristics. Poultry farmers in class 1 were particularly characterized by the diversity of their main activity and their level of education, those in class 2 were mostly employed in agriculture and had little school experience, and those in class 3 were characterized by their low level of vaccination practice and their lack of therapeutic animal care. The results also indicated that 15.7%, 70.8%, and 13.5% of poultry farmers belonged to classes 1, 2, and 3, respectively.
Keywords: Characterization, Farmer, Niger, Poultry diseases, Poultry production

[Full text-PDF]

Review

The Importance of Poultry Meat in Medicine: A Review

Jilo SA and Hasan LA.

J. World Poult. Res. 12(4): 258-262, 2022; pii: S2322455X2200028-12
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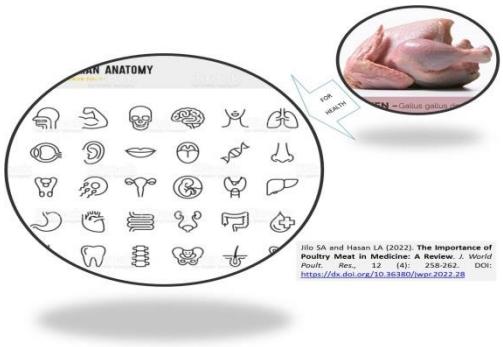

ABSTRACT: The animal products, such as meat, milk, skin, blood, honey, and urine, have medicinal value for human diseases. Due to having high-quality components, poultry meat has therapeutic value. The present review aimed to describe the medicinal values of poultry meat for individuals who consume it during their life. Most poultry meat is classified as white meat, which contains lower fat and higher protein, compared with the meat of ovine, bovine, and pig. This feature of poultry meat (lower fat and higher protein) helps its consumers to have a normal physiological function of different organ systems. Moreover, it prevents many non-infectious diseases, including overweight, diabetes, and cardiovascular diseases. Selenium and low contents of carcinogenic substances (myoglobin, heme iron, and saturated fat) in poultry meat also prevent different types of cancers. Poultry meat is also recommended to avoid anemia, cardiovascular diseases, and diabetes. Dietary proteins, vitamins, and minerals in chicken meat are used for anti-aging, developing muscle and bone, improving the immune system, and increasing brain function. Traditionally, poultry is recommended as a supportive treatment for respiratory diseases, such as the common cold. Thus, consumption of poultry meat, especially chickens, up to 300g/once a week is recommended to prevent and reduce the risks of gastrointestinal cancers such as oesophageal cancer. Generally, regular consumption of poultry meat has health benefits for humans to prevent and reduce the risk of different diseases as chicken meat is a rich source of nutrition that can enhance the immunity system and tackle human disease risk factors.
Keywords: Consumption, Health benefits, Meat, Poultry

[Full text-PDF]

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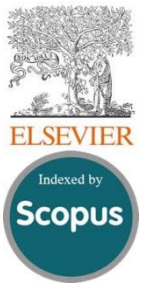

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Effects of a Multi-genus Synbiotic (PoultryStar® sol) on Gut Health and Performance of Broiler Breeders

Zoi Prentza[1], Francesco Castellone[2], Matteo Legnardi[3]*, Birgit Antlinger[4], Maia Segura-Wang[4], Giorgos Kefalas[5], Paschalis Fortomaris[6], Angeliki Argyriadou[6], Nikolaos Papaioannou[7], Ioanna Stylianaki[7], Giovanni Franzo[3], Mattia Cecchinato[3], Vasileios Papatsiros[8], and Konstantinos Koutoulis[1]

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ABSTRACT

In recent years, a rising interest has been directed towards the use of nutraceuticals in the zootechnical sector, including probiotics, prebiotics, and synbiotics, as a way to support production efficiency and cope with the increasing limitations to the use of antibiotics. In poultry, however, most studies on these products have been conducted on broilers, while less information is available on their benefits to other productive categories. The present field study aimed to assess the effects of a multi-species synbiotic product (PoultryStar® sol) on the gut health and productive performance of broiler breeders. A total of 24761 day-old Ross 308 parent stock chicks were acquired from a single hatchery and placed on the same farm. Female chicks were divided into three groups and raised in different houses (A, B, and C), in which males were introduced at the age of mating and followed until 40 weeks of age. The synbiotic was provided by drinking water to the flocks in houses A and B, while house C was kept as control. Following the manufacturer's guidelines, the product was administered intermittently once every two weeks, except in the first and the twenty-first week when it was supplied for three consecutive days. Data on performance parameters, egg quality traits, bacterial enteritis scoring, intestinal morphometry, and histopathology were recorded, and the caecal content was collected at 15, 25, and 40 weeks of age to investigate the intestinal microbiota using high-throughput next-generation sequencing. Synbiotic-treated hens showed significantly higher survivability during production compared to the control group. No clear differences were observed between treated and control chickens in terms of egg production and quality, and the effect of the synbiotic on weight gain also appeared limited. From 25 weeks onwards, synbiotic-treated chickens scored better in terms of macroscopical lesions and had longer intestinal villi. Significant differences in crypt length and histopathological lesions were also found at multiple sampling points. A treatment effect on caecal bacterial composition was detected with a differential abundance of *Gastranaerophilales*, *Lachnospiraceae*, *Helicobacter*, *Ruminococcaceae*, and *Clostridia*, among others. Taken together, obtained results support the beneficial effects of the intermittent administration of the synbiotic product PoultryStar® sol on the gut health of broiler breeders.

Keywords: Broiler breeder, Gastrointestinal health, Histopathology, Microbiota, Synbiotic

INTRODUCTION

The poultry industry is a crucial source of high-quality protein worldwide, with 199 million tonnes of chicken meat produced in 2020 (more than any other meat type) and egg production also accounting for 86 million tonnes (FAOSTAT, 2022). The unceasing growth of the sector is built upon production efficiency, pursued through genetic selection and rigorous health, nutrition, and production

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management. These measures became even more important in recent years due to the emergence of significant new challenges to the profitability and sustainability of the poultry supply chain (Mottet and Tempio, 2017; Hafez et al., 2020).

One of the key areas of interest for the poultry industry is the optimum utilization of available feed ingredients and improvements in nutrient availability (Carré et al., 2008). The intestinal health of poultry plays a role not only in the uptake of nutrients, but also in many aspects of physiology and immune response, with broad implications for animal wellbeing, production efficiency, food safety, and environmental impact (Oviedo-Rondon, 2019). Chicken gut microbiota is known to play a role in the modulation of the host's physiological functions and homeostasis, mainly through the competitive exclusion of detrimental microorganisms and pathogens (Diaz Carrasco et al., 2019). The application of 16S rRNA gene sequencing also revealed the association between enteric dysbiosis and diseases in poultry (Yang et al., 2022). For these reasons, and to cope with the increasing restrictions on the use of antibiotics, a rising interest is paid to nutraceuticals, which are seen as a potential alternative to support production performance (Alagawany et al., 2021). In particular, an ever-growing literature has been produced on probiotics, and their combinations, defined as synbiotics (Awad et al., 2009; Madej et al., 2016; Alagawany et al., 2021).

The efficacy of synbiotics relies on a synergistic effect between probiotics and prebiotics, selectively favoring the survival, implantation, and growth of beneficial bacteria populations in the gut (Awad et al., 2009; Babazadeh et al., 2011; Papatsiros et al., 2013; Nikpiran et al., 2013; Vahdatpour and Babazadeh, 2016; Alizadeh et al., 2017; Syed et al., 2020). Their capacity to improve body weight (BW) gain and feed efficiency (Mousavi et al., 2015; Luoma et al., 2017; Kridtayopas et al., 2019), modulate the immune system and stimulate the development of the gut-associated lymphoid tissue (GALT) and other lymphoid organs (Madej et al., 2015; Madej and Bednarczyk, 2016), and increase the resistance to heat stress (Yan et al., 2019; Jiang et al., 2020; Hu et al., 2022) has been consistently documented. In addition, synbiotics may help to decrease the intestinal and carcass load of various harmful bacteria, including *Campylobacter* (Baffoni et al., 2017), *Clostridium perfringens* (Abd El-Ghany et al., 2010; Shanmugasundaram et al., 2020) and *Salmonella enterica* serovar *Enteriditis* (Markazi et al., 2018; Shanmugasundaram et al., 2019; Sobotik et al., 2021).

Since most of the experiments on synbiotics have been conducted in broilers, less is known about their possible applications in other productive categories, whose different genetic features and farming systems entail different challenges and requirements. For this reason, this study aimed to evaluate the benefits of a multi-species synbiotic product on broiler breeders, by assessing its effects on performance and gut health during the rearing and laying periods.

MATERIALS AND METHODS

Ethical approval

Ethical review and approval were waived for this study since animals were sampled during commercial activities in the farm regulated by national and international laws.

Experimental design

The present field study was conducted in a private broiler breeder farm located in the region of Ioannina, Greece, and covered the first 40 weeks of age of the chickens. A total of 24761 day-old Ross 308 parent stock chicks were supplied from the same hatchery and placed in separate houses on the same farm. In detail, 6200, 6264, and 8937 females were placed in houses A, B, and C, respectively. The synbiotic was administered to houses A and B, while house C acted as a control group. A total of 3360 males were raised in a separate house and were introduced in houses A, B, and C at the age of mating (19 weeks) with a ratio of one male to 10 females.

Management

To ensure flock welfare and achieve high performance, management conditions followed the official guidelines for parent stocks (Aviagen, 2018). Chickens were placed on a floor covered with straw (deep litter system) and were fed *ad libitum* for the first 2 weeks. Restricted daily feeding was observed from the second to the fourth week; then, starting from week 4, the feed was supplied on a skip-a-day regimen. Feed allocation followed the recommendations for breeders, weighing the chickens weekly and adjusting the dose accordingly (Aviagen, 2018). The light period was 20 hours in the first week, 12 hours in the second week, and 8 hours from the third to week 21. From week 21 onwards, the light period was increased from 8 hours up to 14 hours based on average BW and weight uniformity. The temperature was set according to official guidelines, starting at 30°C at the chicks' arrival and decreasing by 1°C every three days

until day 27, then keeping it at 20°C for the rest of the productive cycle. The relative humidity was kept at 60-70% (Aviagen, 2018). Stocking densities were seven female chickens/m^2 and five male chickens/m^2, as indicated by EFSA (2010).

The diet was formulated in accordance with the official genetic line guidelines (Aviagen, 2016), implementing a seven-phase feeding system (starter 1, 0-21 days; starter 2, 22-35 days; grower, 36-105 days; pre-breeder, 106 days to 5% production; breeder 1, 5% production to 245 days; breeder 2, 246-350 days; breeder 3, after 351 days). The exact nutrient specifications are provided in Table 1. Water was provided *ad libitum.*

Chickens were vaccinated at the hatchery against infectious bursal disease (IBD) and Marek's disease (MD). The full vaccination protocol was administered throughout the cycle, including vaccines against infectious bronchitis (IB), Newcastle disease (ND), avian rhinotracheitis (ART), chicken infectious anemia (CIA), infectious avian encephalomyelitis, *Escherichia coli*, salmonellosis, and coccidiosis (Table 2). No antibiotics were administered throughout the considered period.

Table 1. Nutrient composition of the seven-phase feeding system observed to raise the Ross 308 broiler breeders used in the experiment

Diet	**Starter 1 (days 1-21)**		**Starter 2 (days 22-35)**		**Grower (days 36-105)**		**Pre-Breeder (day 106 to 5% production)**		**Breeder 1 (5% production to day 245)**		**Breeder 2 (days 246-350)**		**Breeder 3 (after day 351)**	
Energy	2800 kcal/kg		2800 kcal/kg		2600 kcal/kg		2700 kcal/kg		2800 kcal/kg		2800 kcal/kg		2800 kcal/kg	
Amino acids (%)	**Total**	**Digest**	**Total**	**Digest**	**Total**	**Digest**	**Total**	**Digest**	**Total**	**Digest**	**Total**	**Digest**	**Total**	**Digest**
Lysine	1.06	0.95	0.74	0.67	0.58	0.52	0.58	0.52	0.67	0.60	0.62	0.56	0.58	0.52
Methionine + Cysteine	0.84	0.74	0.67	0.59	0.59	0.52	0.58	0.51	0.67	0.59	0.65	0.57	0.59	0.54
Methionine	0.51	0.46	0.41	0.37	0.36	0.33	0.35	0.32	0.41	0.37	0.40	0.36	0.36	0.35
Threonine	0.75	0.66	0.60	0.53	0.50	0.44	0.47	0.41	0.55	0.49	0.53	0.47	0.51	0.47
Valine	0.80	0.71	0.70	0.63	0.49	0.44	0.51	0.45	0.63	0.56	0.60	0.53	0.57	0.51
IsoLeucine	0.70	0.62	0.62	0.55	0.45	0.40	0.47	0.41	0.56	0.50	0.54	0.48	0.51	0.45
Arginine	1.17	1.05	0.93	0.83	0.71	0.64	0.74	0.67	0.88	0.79	0.86	0.77	0.80	0.72
Tryptophan	0.19	0.16	0.18	0.15	0.14	0.12	0.15	0.13	0.16	0.14	0.15	0.13	0.14	0.12
Leucine	1.23	1.11	0.93	0.83	0.77	0.69	0.80	0.72	1.04	0.94	1.00	0.90	0.96	0.86
Crude Protein	19.00		17.00		13.00-14-00		14.00		15.00		14.00		13.00	
Minerals (%)														
Calcium	1.00		1.00		0.90		1.20		3.00		3.20		3.40	
Available Phosphorus	0.45		0.45		0.42		0.35		0.35		0.33		0.32	
Sodium	0.18-0.23		0.18-0.23		0.18-0.23		0.18-0.23		0.18-0.23		0.18-0.23		0.18-0.23	
Chloride	0.18-0.23		0.18-0.23		0.18-0.23		0.18-0.23		0.18-0.23		0.18-0.23		0.18-0.23	
Potassium	0.40-0.90		0.40-0.90		0.40-0.90		0.60-0.90		0.60-0.90		0.60-0.90		0.60-0.90	
Added trace minerals (mg/kg)														
Copper	16								10					
Iodine	1.25								2.00					
Iron	40								50					
Manganese	120								120					
Selenium	0.30								0.30					
Zinc	110								110					
Minimum specifications														
Choline (mg/kg)	1400		1400		1300		1200		1200		1050		1050	
Linoleic acid (%)	1.00		1.00		1.00		1.00		1.25		1.25		1.25	
Added Vitamins/Kg	**Wheat-based feed**				**Maize based feed**				**Wheat-based feed**				**Maize based feed**	
Vitamin A (IU)	11000				10000				12000				11000	
Vitamin D3 (IU)	3500				3500				3500				3500	
Vitamin E (IU)	100				100				100				100	
Vitamin K (mg)	3				3				5				5	
Thiamin (B1) (mg)	3				3				3				3	
Riboflavin (B2) (mg)	6				6				12				12	
Nicotinic Acid (mg)	30				35				50				55	
Pantothenic Acid (mg)	13				15				13				15	
Pyridoxine (B6) (mg)	4				3				5				4	
Biotin (mg)	0.20				0.15				0.30				0.25	
Folic Acid (mg)	1.50				1.50				2.00				2.00	
Vitamin B12 (mg)	0.02				0.02				0.03				0.03	

Table 2. Vaccination protocol administered at the hatchery and throughout the production cycle on the Ross 308 broiler breeders used in the experiment

Age (day)	Vaccine(s)	Disease(s)	Route
day 18 of incubation	Cevac® MD HVT+Rispens	Marek's disease	*In ovo* injection
Hatch day	Cevac® Transmune IBD	Infectious bursal disease virus	Subcutaneous injection
1	Nobilis® IB H120 + Cevac® IBird + Poulvac® E. coli	Infectious bronchitis + colibacillosis	Spray
2	Gallivac® Se + AviPro Salmonella VAC T	Salmonellosis (*Salmonella enteriditis and Typhimurium*)	Water
6	Paracox®	Coccidiosis	Spray/Water
10	Avinew®	Newcastle disease	Spray/Water
18	Nobilis® IB 4/91	Infectious bronchitis	Spray/Water
28	Nobilis® IB Ma5+ Nobilis® ND Clone 30	Infectious bronchitis + Newcastle disease	Spray/Water
35	Nemovac	Avian rhinotracheitis	Spray
50	Gallivac® Se + AviPro Salmonella VAC T	Salmonellosis (*Salmonella Enteriditis* and *Typhimurium*)	Water
55	Avinew®	Newcastle disease	Spray/Water
70	Nemovac	Avian rhinotracheitis	Spray
78	Nobilis® IB Ma5 + Nobilis® ND Clone 30	Infectious bronchitis + Newcastle disease	Spray/Water
88	Nobilis® IB 4/91	Infectious bronchitis	Spray/Water
92	AviPro Thymovac®	Chicken infectious anemia	Water
100	Nobilis® ND Clone 30+ Poulvac® *E. coli*	Newcastle disease + colibacillosis	Spray
107	AviPro AE®	Infectious avian encephalomyelitis	Water
125	Gallimune® 303 + Gumboriffa® + Gallimune® SE+ST + Hiprapox®	Newcastle disease + infectious bronchitis + avian rinotracheitis + infectious bursal disease + salmonellosis (*Salmonella Enteriditis* and *Typhimurium*)+ fowlpox	Intramuscular injection- wing web stab
154	Avinew®	Newcastle disease	Water
224	Nobilis® IB Ma5 + Avinew®	Infectious bronchitis, Newcastle disease	Water

Synbiotic administration

The synbiotic product PoultryStar® sol (BIOMIN GmbH, Getzersdorf, Austria), containing patented probiotic strains plus prebiotic fructooligosaccharides, was applied in houses A and B by drinking water based on a protocol planned with the manufacturer's guidance. In detail, a daily dosage of 20 g/1,000 chickens was supplied for three consecutive days during weeks 1 and 21 (the first administration after males were introduced) and for one day every two weeks during the rest of the cycle of the product.

Sample collection

Ten randomly selected chickens per treatment group were euthanized by cervical dislocation at 15, 25, and 40 weeks of age to collect specimens for histopathological analysis and lesion scoring. About 3 g of caecal content was also collected to evaluate the microbial composition.

Performance parameters

Live BW and mortality were recorded on a weekly and daily basis, respectively, and egg production was expressed on a hen-day basis from the beginning of the production period (23 weeks) up to 40 weeks. Egg fertility and hatchability were recorded as a percentage of total settable eggs throughout the laying period.

Egg quality traits

At week 30, from the beginning of the laying period, 20 eggs per group were randomly collected every two weeks up to week 40 to assess several external and internal egg traits. Individual eggs were weighed to the nearest 0.01 g accuracy with a digital balance, and the egg length and breadth were measured using digital calipers. A shape index was then calculated by dividing the breadth by the length and multiplying by 100. The shell strength was measured using TA.HD plus Texture Analyser (Stable Micro Systems Limited, Godalming, UK). Shell weight

was measured after removing the inner shell membrane and keeping it dry for 24 hours. Shell thickness was evaluated using the Egg Shell Thickness Measure Model 25-5 (B.C. Ames Incorporation, Melrose, Massachusetts) by considering the average of three equidistant points on the equator. The albumen height was measured with the Egg Quality Micrometers S-8400 spherometer (B.C. Ames Incorporation, Melrose, Massachusetts) at 3-4 locations and averaged. The yolk and albumen were weighed to the nearest 0.01 g accuracy on a digital balance. The Haugh unit (HU) was calculated using the formula HU = 100 logs (H+7.57-1.7 W37), where H is the height of the albumen in millimeters and W is the egg weight in grams.

Bacterial enteritis scoring

A macroscopic lesion scoring system was applied to evaluate the chickens' intestinal health in each group at three different time points. Specifically, ten parameters (De Gussem, 2010) were assessed by visual inspection of the intestinal wall during the necropsy. Each parameter was scored 0 when absent and 1, summed and divided by 2.5, resulting in a total score between 0 (normal gastrointestinal tract) and 4 (severe dysbacteriosis) (De Gussem, 2010; Teirlynck et al., 2011).

Histology

Segments of 3 cm were collected from the duodenum, jejunum, ileum, and caecum, keeping the collection sites consistent for each tract. All samples were placed in individually labeled flasks containing 10% neutral buffered formalin, as described by Hoerr (2001). Transversal sections approximately 1 mm thick of each sample were then cut after 48 hours. Sections of 3-5 μm were taken, stained with hematoxylin and eosin, and evaluated. The histopathological and morphometrical evaluation of specimens was performed blindly. The scoring system proposed by Kraieski et al. (2017) was adopted to assess the degree of inflammation in each section. Specifically, the severity of the lesions was graded on a 0-3 scale: 0 corresponded to absent or rare leukocytic infiltration, 1 to leukocytic infiltration up to 5% of a field at x400, 2 to approximately 25% leukocytic infiltration of a field at the same magnification, 3 to leukocytic infiltration in the range of 50%. The morphometry of the intestinal villi and crypts was examined using optical capture and measurement with Image Pro-Plus version 6.0 software (Media Cybernetics, Silver Spring, MD). The selection of the villi for the morphometrical analysis was conducted according to Gava et al. (2015), considering only those that had their bases embedded in the submucosa, without any discontinuity or folds in their length, and with intact epithelium at the tip.

Evaluation of enteric microbiota

High-throughput sequencing was performed on a total of 64 samples, consisting of 10 caecal content from each treatment group. For each sampling point, two meconium samples from the breeders' grandparents (sequencing controls) and two water samples (contamination controls). The analysis was performed on an Illumina MiSeq System (Illumina, San Diego, California) at BioLizard (Ghent, Belgium), LGC genomics (Berlin, Germany) targeting the V3 region of the 16S rRNA gene, and generated 2 x 300 paired-end sequences. Following a preliminary evaluation of the read quality of unmerged sequences with FastQC 0.11.9, the forward reads were trimmed at 195 bp, and the reverse reads at 220, ensuring a minimal Phredscore of 28. The amplicon sequence variants (ASVs) that most accurately describe the data were inferred with DADA2 (Callahan et al., 2016), and then the forward and reverse reads were merged, setting the minimal overlap to 12 bp. After removing chimeric sequences from the dataset, the SILVA 138 reference database (Quast et al., 2013; Yilmaz et al., 2014) was used to classify ASVs as taxons.

Four diversity indexes (Simpson, Shannon, Chao1, and Observed species index) were used to calculate the alpha diversity. Permutational ANOVAs were performed on the euclidean distances between samples for significance testing between groups. Since these tests require an adequate homogeneity of the separate group dispersions, this assumption was first verified with the betadisper function from the vegan R package (Dixon, 2003). To verify the presence of no systematic biases or confounding effects, the Spearman correlation of the treatment effect with other variables (such as age, weight, bacterial enteritis score, histological lesion scores, crypts, villi length, etc.) was run. Differential abundance analysis was then performed with DESeq2 to evaluate the isolated effect of the treatment and the other factors.

Statistical analysis

Data were organized and analyzed in R version 3.3.2 (R Core Team, 2013). For each considered variable, the statistical significance of between-treatment differences was evaluated at each time point using a Student t-test or, if relative assumptions were violated, the non-parametric Mann-Whitney test. Differences between the three houses were evaluated using ANOVA or, in case the relative assumptions were not met, with the Kruskal-Wallis test

followed by post-hoc Mann-Whitney test with Bonferroni correction. Survival analysis was performed using the survival library in R. Kaplan-Meier cumulative survival curves were calculated, and the significance of the difference between treatment groups in the survival curves was assessed using the Log-rank (M-H). The significance level was set to $p < 0.05$. The statistical evaluation of sequencing data was performed independently at BIOLIZARD NV (Ghent, Belgium). For differential abundance analysis, the significance level was set to $p < 0.01$.

RESULTS

Bacterial enteritis and histopathological lesion scores

The BE score measured in the control group was higher than in the treated chickens at every time point, with a statistically significant difference ($p = 0.049$) observed at week 25 (Graph 1). No significant differences were found between houses. As for the histopathological lesion score, lower and statistically significant scores were found in the synbiotic-treated chickens than in control ones at week 25 in the caecum ($p = 0.025$), and at week 40 at caecum ($p = 0.021$) and ileum ($p = 0.002$). Conversely, the control group showed a lower score than treated chickens in the jejunum at week 25 ($p = 0.032$, Graph 2). No significant differences ascribable to the house effect were found at between the two treatment houses at duodenum level at week 15 ($p = 0.42$), week 25 ($p = 0.6$) and week 40 ($p = 0.18$); at jejunum level at week 15 ($p = 0.42$), week 25 ($p = 0.6$) and week 40 ($p = 1$); at ileum level at week 15 ($p = 0.42$), week 25 ($p = 1$) and week 40 ($p = 0.27$); and at caecum level at week 15 ($p = 0.42$), week 25 ($p = 0.27$) and week 40 ($p = 0.42$).

Evaluation of intestinal villi and crypts

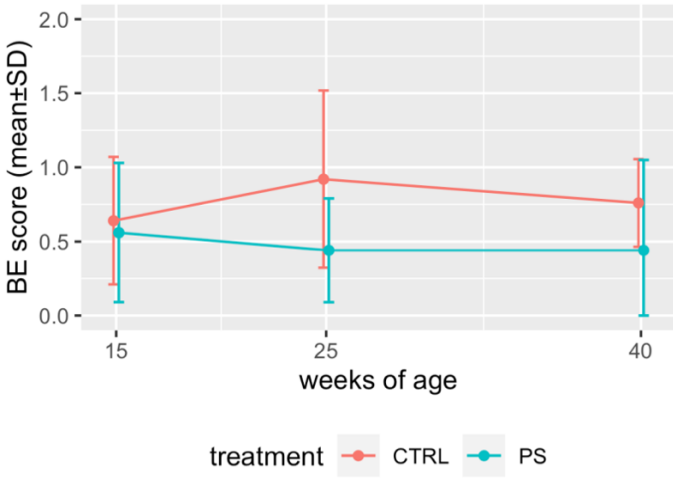
As shown in Graph 3, several differences could be observed between treated and control animals in terms of gut morphometric parameters. Considering only significant differences, synbiotic-treated chickens showed longer villi than control chickens at week 15 in the ileum ($p = 0.004$), at week 25 at the duodenum ($p < 0.0001$), jejunum ($p < 0.0001$), ileum ($p = 0.001$) and caecum ($p < 0.0001$) level, and again at week 40 in all four tracts (all with $p < 0.0001$). Less consistent differences were observed when measuring the crypts, which were significantly deeper in synbiotic-treated than in control chickens in the duodenum at week 25 ($p < 0.0001$) and in the jejunum tract at week 15 ($p < 0.0001$) and week 40 ($p = 0.0004$), but less deep in the caecum at week 25 ($p = 0.002$). The house effect on villi length was significant in the duodenum at week 15 ($p = 0.005$), in the jejunum at week 25 ($p < 0.0001$) and week 40 ($p = 0.009$), in the ileum at week 40 ($p = 0.006$) and in the caeca at week 25 ($p = 0.007$). In terms of crypt length, houses A and B differed significantly at week 25 at the duodenum ($p < 0.0001$) and jejunum level ($p = 0.006$, Graph 4).

Performance

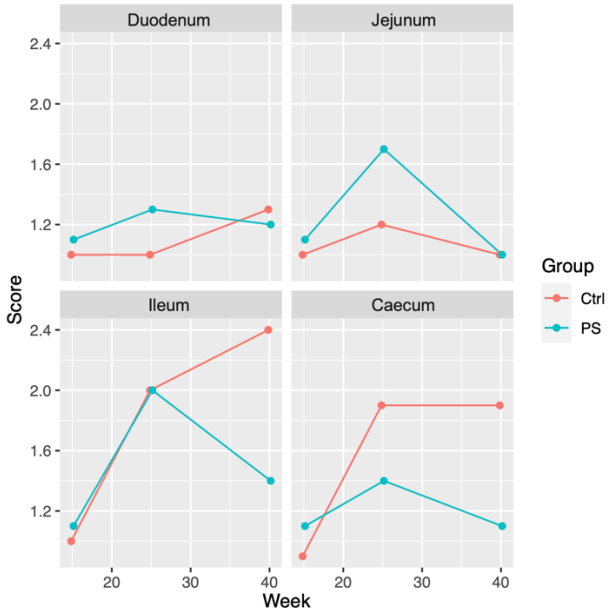
There was a significant between synbiotic-treated chickens and the control group in terms of live BW, (Graph 5, $p = 0.05$). However, the house effect seemed far more relevant in determining the observed differences ($p < 0.0001$), as house C (control) performed better than house B but worse than house A. In particular, the biggest difference was observed in the BW of males, which was remarkably higher for house A ($p < 0.0001$ when compared to both houses B and C). On the other hand, the BW of producing hens was less heterogeneous, and better performance was observed in house C than in the treated houses ($p < 0.001$ for both comparisons, Graph 5b). A significant difference in terms of survivability throughout the production period (23-40 weeks) was observed between the treated and control groups ($p < 0.001$) (Graph 6a). Significant differences were also observed when considering the three houses separately ($p < 0.001$), with both treatment houses scoring better than the control (Graph 6b). No significant differences were found in terms of egg fertility and hatchability, neither between synbiotic-treated and control chickens ($p = 0.12$ for egg fertility, $p = 0.67$ for hatchability) nor between treated houses ($p = 0.1$ for egg fertility, $p = 0.47$ for hatchability).

Egg quality traits

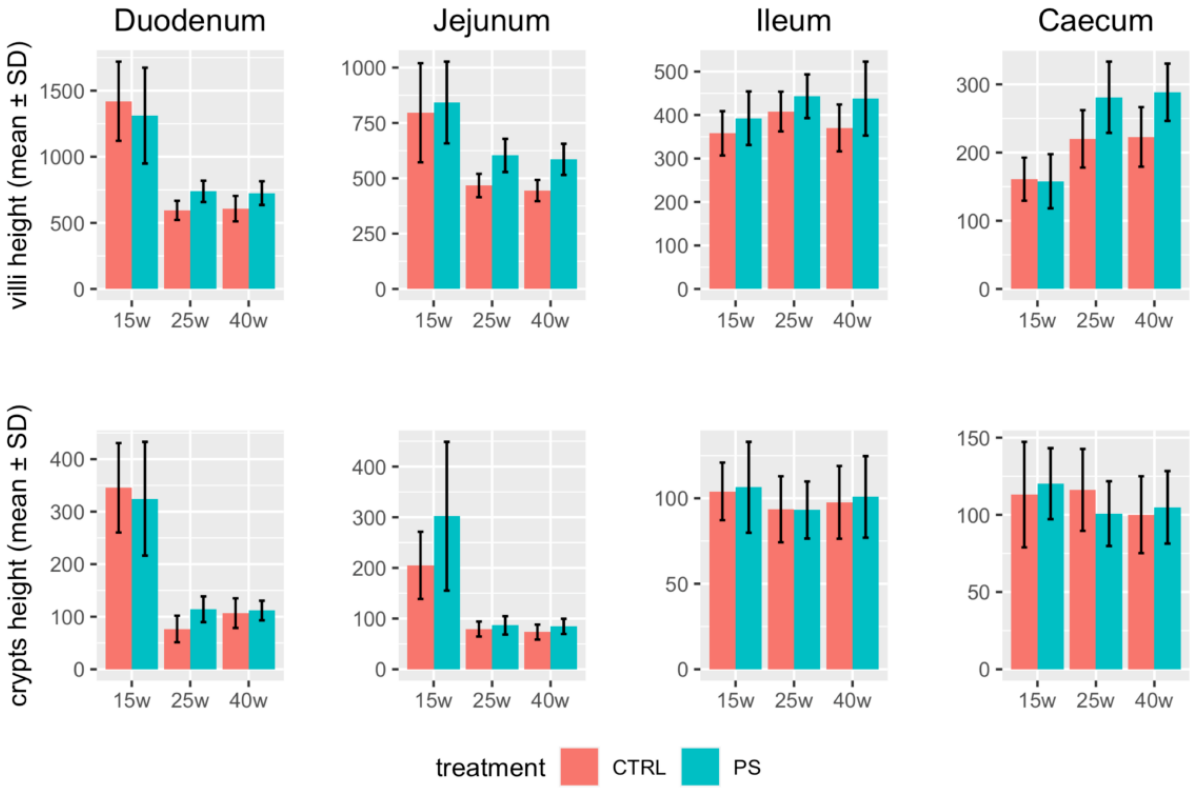
There were no significant differences in terms of eggshell strength, shell thickness, and shape index, but some were found at limited time points in egg weight, shell weight, and combined albumen and yolk weight between treatments and, more limitedly, between houses. In particular, the egg weight was higher in synbiotic-treated chickens than in control ones at week 30 ($p = 0.009$) but lower at week 40 ($p = 0.032$). Shell weight was higher in synbiotic-treated chickens than in control ones at week 30 ($p = 0.018$). The combined weight of yolk and albumen was higher in control chickens than in synbiotic-treated ones at week 40 ($p = 0.026$). Overall, no clear trends that could be ascribable to the synbiotic treatment were identified (Graph 7).



Graph 1. Bacterial enteritis score measured in synbiotic-treated and control broiler breeders



Graph 2. Histopathological lesion scores measured in different intestinal tracts in synbiotic-treated and control broiler breeders



Graph 3. Gut morphometric parameters measured in different enteric tracts in synbiotic-treated and control chickens

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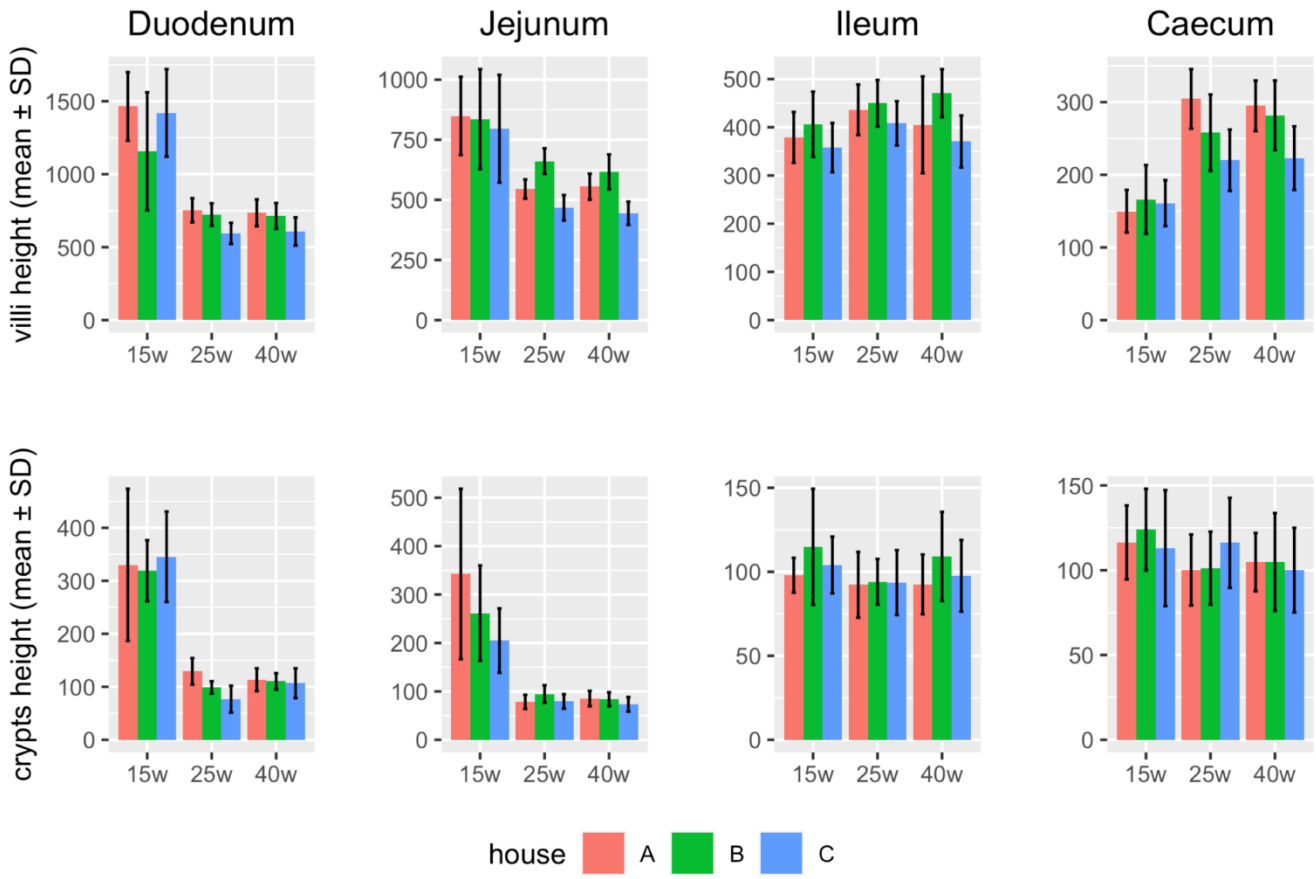
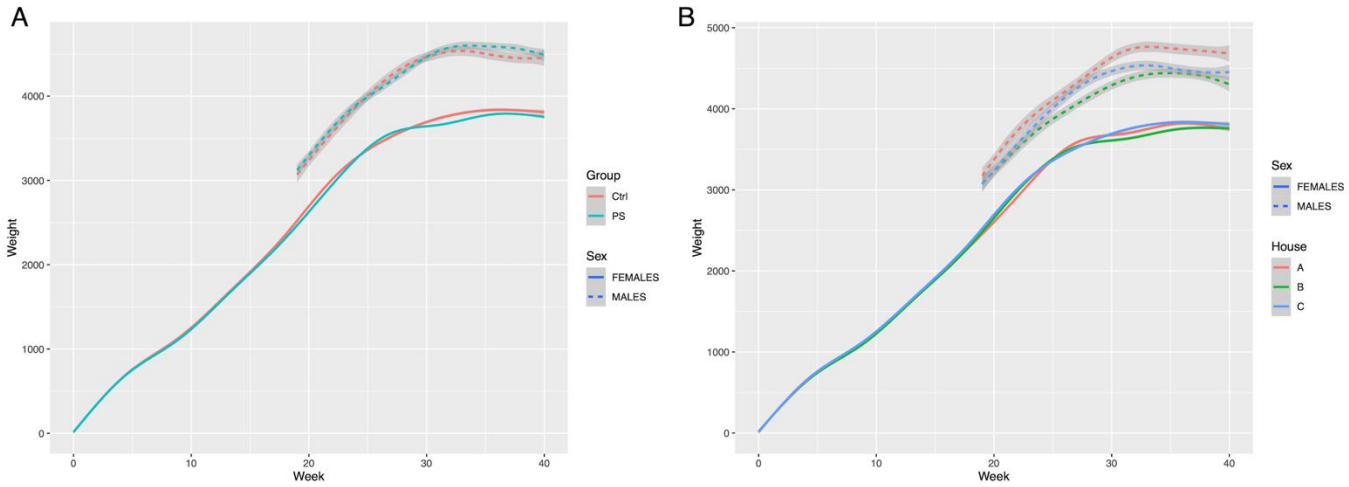
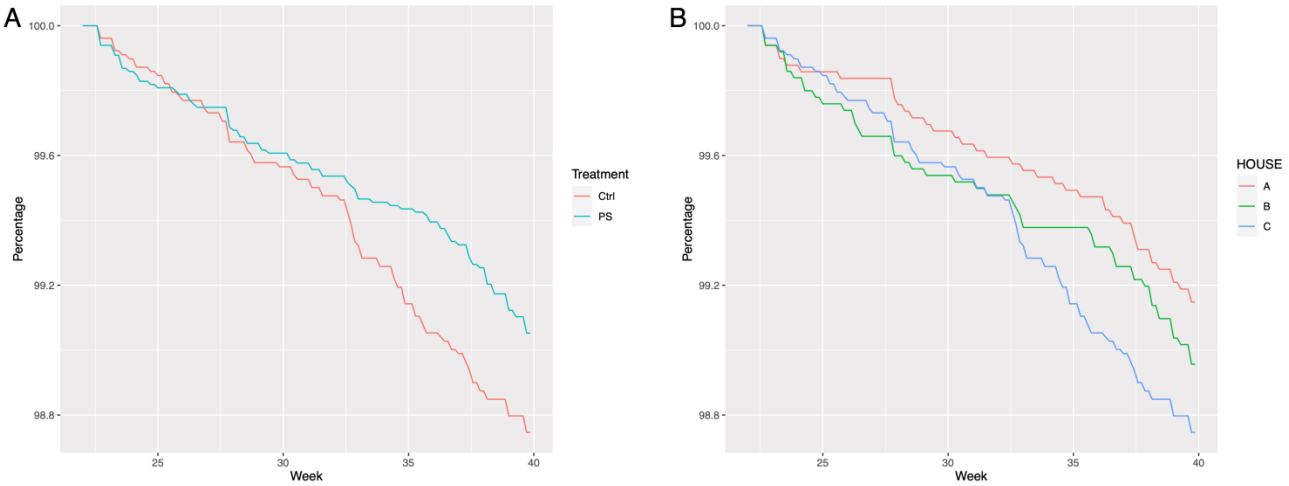


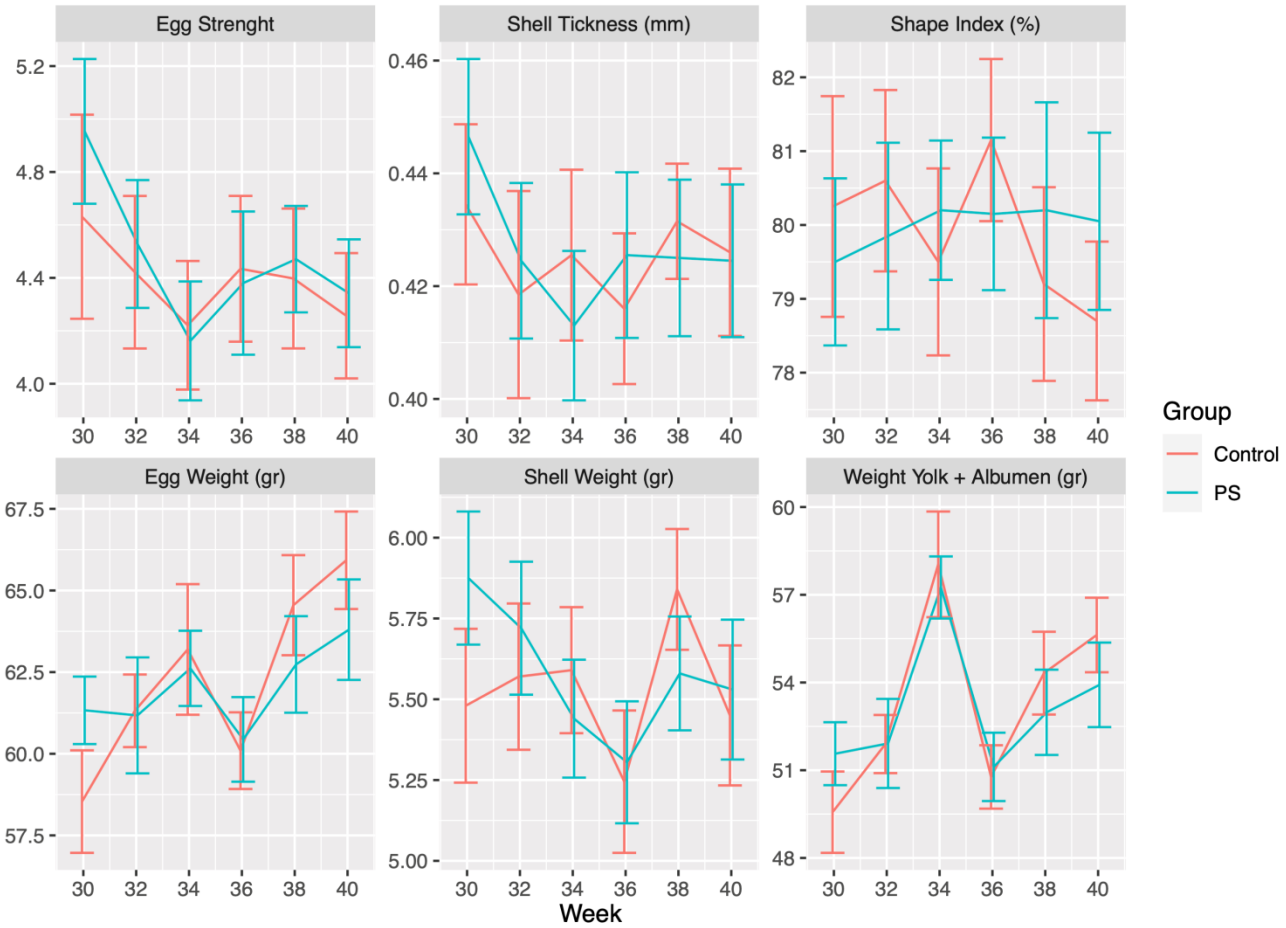
Graph 4. Gut morphometric parameters measured at 15, 25, and 40 weeks of age in different enteric tracts of the broiler breeders raised in the three houses. The synbiotic was administered in houses A and B, while house C acted as the control group



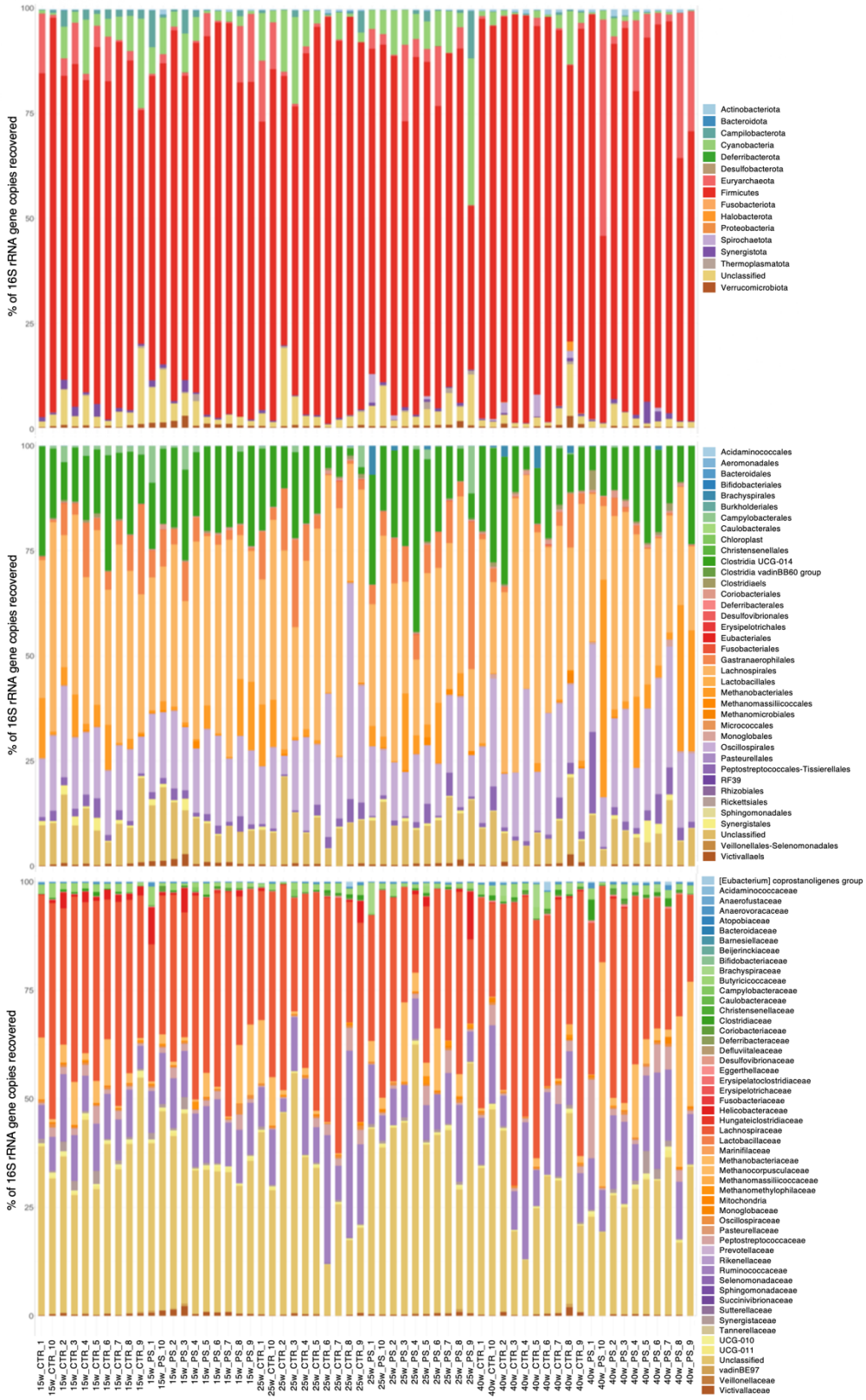
Graph 5. Growth curves comparison between synbiotic-treated and control broiler breeders (a) and between the three houses (b). The synbiotic was administered in houses A and B, while house C acted as the control group



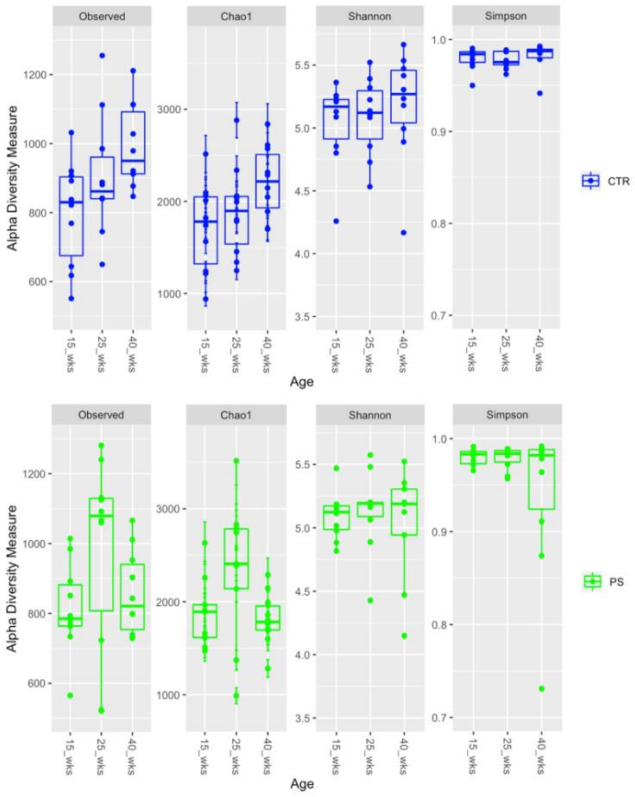
Graph 6. Comparison of survivability rates during the production period (23-40 weeks) between synbiotic-treated and control female broiler breeders (a) and between the three houses (b). The synbiotic was administered in houses A and B, while house C acted as the control group.



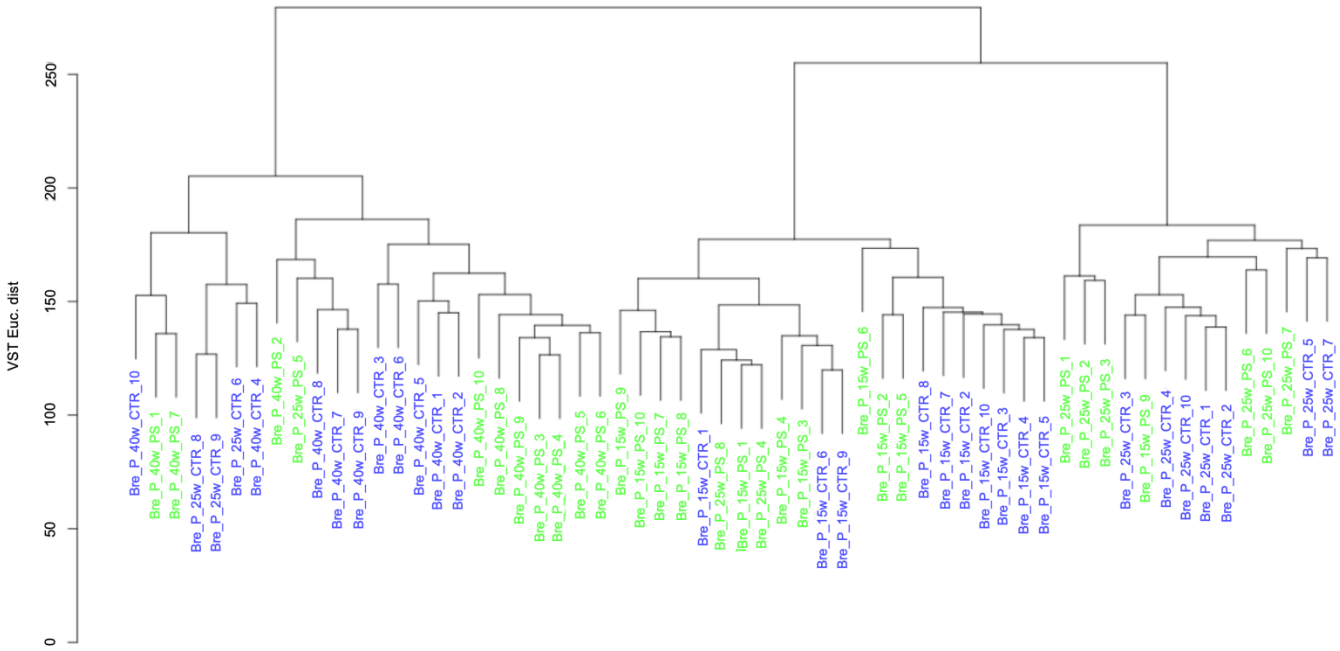
Graph 7. Comparison of egg traits between synbiotic-treated and control broiler breeder chickens



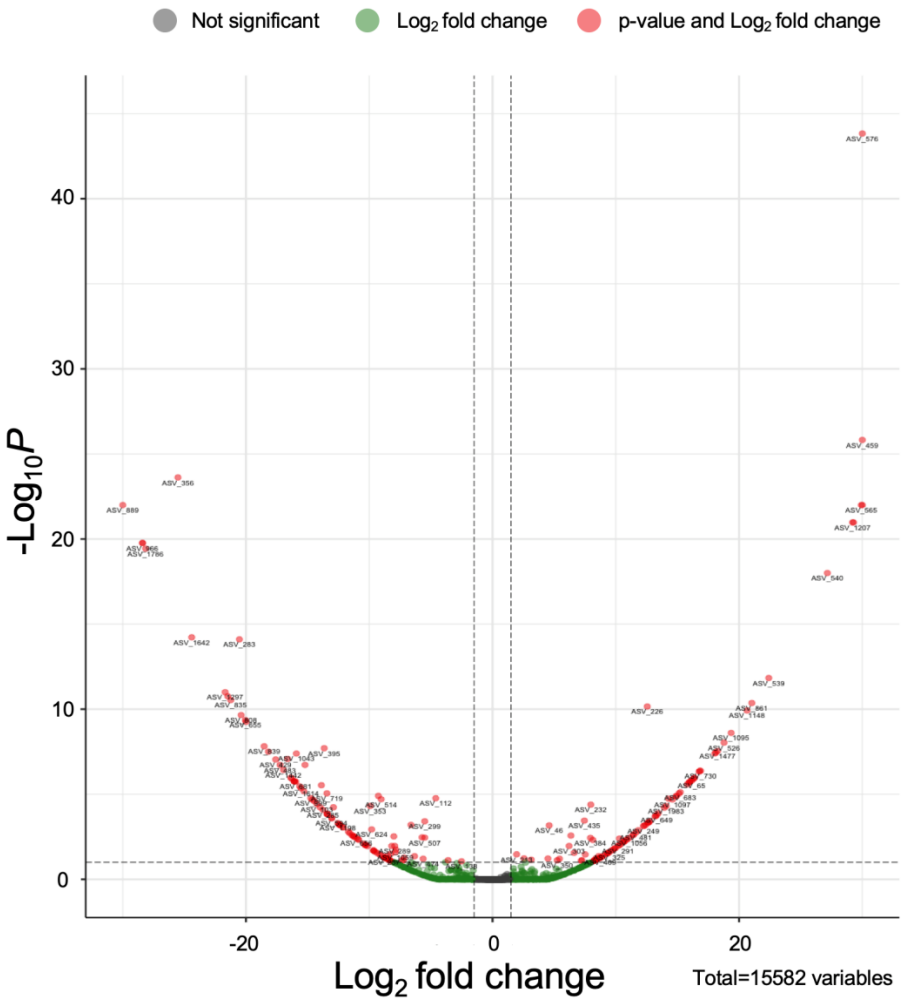
Graph 8. Relative microbial composition of caecal content of synbiotic-treated and control broiler breeder chickens, shown at Phylum (top), Order (centre) and Family (bottom) level.



Graph 9. Alpha-diversity indexes measured in synbiotic-treated (PS) and control (CTR) broiler breeder chickens and divided per age group.



Graph 10. Dendrogram of the broiler breeder caecum samples, clustered on the Euclidean distance between their count data. Sample names are colored green for synbiotic-treated chickens and blue for control chickens. The age at sampling (15, 25 and 40 weeks) is indicated in the code of each sample.



Graph 11. Volcano plot showing the differential abundance of amplicon sequence variants in the caecal microbiota of broiler breeders due to the synbiotic treatment effect. The statistical significance value was set to $p < 0.01$ (horizontal line), while, to be considered biologically significant, the effect size expressed in terms of Fold Change (FC) should have had an absolute value of 3 (vertical lines at $\log_2$ FC = 1.5).

Evaluation of enteric microbiota

According to sequencing results, the overall diversity in the caecum samples was rather high, with a total of 15582 different ASVs. The relative microbial abundance of each caecal content is shown in Graph 8.

According to the measured diversity indexes, the richness of different bacterial species was rather high in most of the samples and generally increased between weeks 15 and 25. A less evident trend was observed from week 25 to 40, when the bacterial diversity in the synbiotic-treated chickens was even shown to decrease (Graph 9).

Hierarchical clustering on euclidean distance showed that samples tended to cluster based on treatment and age, with clear segregation between 15-week-old and 40-week-old chickens and only a slight overlap of 25-week-old chickens with both groups (Graph 10).

A significant treatment effect was found by comparing the microbial composition of samples from synbiotic-treated and control chickens ($p = 0.025$). When the comparisons were between same-age chickens, the treatment effect was significant at week 15 ($p < 0.001$) and week 40 ($p = 0.03$), but not significant at week 25 ($p = 0.064$). The age effect was confirmed significant by comparing samples taken at different ages, both among treated and control chickens ($p < 0.001$ in both cases). Since synbiotic-treated chickens were reared in two separate houses, the possible house effect was also investigated but was found to be non-significant ($p = 0.083$). Intercorrelation analysis revealed no significant

Spearman correlation of any variables to the treatment, indicating a proper experimental setup. When isolating the treatment effect, significant differences were detected in the abundance of 119 out of a total of 15582 ASVs (after Benjamini-Hochberg multiple testing correction, Graph 11). In particular, 45 ASVs were more abundant in the treated breeders, while 74 were less abundant. Among others, the treatment effect seems to have affected the relative abundance of *Gastranaerophilales*, *Helicobacter*, *Ruminococcaceae*, *Lachnospiraceae*, and *Clostridia* (Table 3).

Table 3. Top 10 differentially abundant amplicon sequence variants for the treatment effect ranked on the adjusted p-value. The direction of differential abundance can be inferred from the sign of the Log2 Fold Change

Amplicon sequence variant	Log2 fold change	Standard error	Adjusted p-value	Lowest resolved taxon
ASV_576	30.000000	2.057181	1.4620e-44	Gastranaerophilales
ASV_459	30.000000	2.643062	1.4979e-26	*Helicobacter*
ASV_356	-25.517909	2.349552	2.3997e-24	*Ruminococcaceae*
ASV_565	29.919828	2.863515	1.0061e-22	*Lachnospiraceae*
ASV_797	30.000000	2.870802	1.0061e-22	Bacteria
ASV_889	-29.994918	2.864174	1.0061e-22	Gastranaerophilales
ASV_1207	29.208433	2.864828	1.0554e-21	Clostridia UCG-014
ASV_1822	29.286145	2.872488	1.0554e-21	Clostridia UCG-014
ASV_966	-28.400792	2.867760	1.7165e-20	Clostridia UCG-014
ASV_1298	-28.383064	2.867419	1.7165e-20	Clostridia

DISCUSSION

The present results comprehensively depict the effects of the considered synbiotic product on the performance and gut health of broiler breeders. Following a protocol devised with the manufacturer's guidance, PoultryStar® sol was administered for three consecutive days of weeks 1 and 21, as recommended for newly hatched poultry and around stressful periods and changes, such as the introduction of males. An intermittent schedule was observed throughout the rest of the cycle, which is recommended to support gut eubiosis continuously.

Regarding the obtained results, it is useful to compare them to those obtained in previous trials of other synbiotics, bearing in mind that the outcomes may differ depending on each product's composition, dosage, administration route, and timing, along with environmental and host-related factors.

The effect of PoultryStar® sol administration on BW gain appeared limited, and the observed heterogeneity between the different groups seemed more easily ascribable to the house effect. Several synbiotics, mostly tested on broilers, were shown to increase BW gain and feed conversion ratio (Mohammed et al., 2018; Kridtayopas et al., 2019; Abdel-Wareth et al., 2019), while others had no impact on BW or feed conversion ratio (Chang et al., 2019; Dankowiakowska et al., 2019; Shanmugasundaram et al., 2020). Ultimately, it should also be considered that breeders' feeding programs are targeted at maintaining high weight uniformity and keeping close to BW targets, rather than maximizing growth and feed efficiency (Aviagen, 2018). Any overperformance compared to target BW during both rearing and production periods, may be compensated with feed restrictions (EFSA, 2010), thus masking any potential increase in feed efficiency related to synbiotic administration.

Egg production and quality were also evaluated, as several synbiotics were shown to improve them. Luoma et al. (2017) found that administering a multi-species synbiotic increased egg production between 19 and 28 weeks of age, even after the chickens were challenged with *Salmonella enterica* serovar Enteritidis. Similar results were obtained by Radu-Rusu et al. (2010), Abdel-Wareth (2016), and Tang et al. (2017), who also reported a positive effect on egg quality, resulting in heavier, larger eggs with thicker shells. According to Buyarov and Metasova (2019), synbiotic-fed broiler parent stocks also showed an increase in egg production and hatchability. On the other hand, other tested probiotics and synbiotics had limited or no effect on laying performance (Tang et al., 2015; Liu et al., 2019; Sjofjan et al., 2021). In the present study, no significant differences were found in terms of egg fertility, hatchability, and morphology, except for specific sampling points in terms of egg weight, shell weight, and combined albumen and yolk weight. Based on

these findings, the tested synbiotic did not seem to affect egg production.

A significant treatment effect was found in terms of survivability during the laying period, with both treated groups exhibiting lower mortality than the control one. The decision to focus on the production phase was taken because mortality rates in the rearing phase may be easily altered by culling procedures, which are often due to factors unrelated to the breeders' health, such as chickens not meeting selection criteria or sexing errors (EFSA, 2010). The observed differences suggest that PoultryStar® sol can effectively reduce mortality in field conditions, as already reported for other synbiotics (Awad et al., 2009; Abdel-Wareth et al., 2019; Rodrigues et al., 2020).

Although the ultimate goal of synbiotic administration is to have healthier and, thus, more productive chickens, the evaluation of performance parameters only offers a partial and indirect assessment of their effect on gut health. Ringenier et al. (2021) noted that a healthier intestinal tract does not always correspond to an increase in production parameters, as birds can cope with a certain degree of gut lesions before their performance is affected. For this reason, gut health scores and intestinal morphometry were also considered to assess the effect of PoultryStar® sol in preventing any unfavorable state of inflammation or dysbacteriosis which could negatively alter the integrity of the intestinal mucosa and thus its absorption and immune functions (Willing and Van Kessel, 2009; Teirlynck et al., 2011).

The BE score was lower in treated chickens than in control ones at all time points, with a statistically significant difference at 25 weeks of age. The histopathological lesion score was also significantly lower in the treated groups in the caecum (at 25 and 40 weeks) and ileum (at 40 weeks), while the control group scored better only at a single point at the jejunum level. According to these results, synbiotic-treated chickens exhibited better intestinal health even in the absence of a challenge. This conclusion is supported by the evaluation of gut morphometric parameters, which showed that synbiotic-treated chickens had longer villi consistently along all intestinal tracts from 25 weeks of age onwards. Synbiotic trials often report an increase in villus height in different intestinal tracts, indicating a larger surface for nutrient absorption (Samanya and Yamauchi, 2002) throughout different intestinal tracts (Kridtayopas et al., 2019; Villagrán-de la Mora et al., 2019; Jiang et al., 2020). The effect of PoultryStar® sol on crypts, whose depth is related to the mucosal proliferative activity (Prakatur et al., 2019), appeared less evident and consistent, with deeper crypts being reported in the jejunum and duodenum, while caecal crypts were less deep at 25 weeks of age. Similar findings are reported in previous studies, in which different synbiotic formulations were shown to increase (Villagrán-de la Mora et al., 2019), decrease (Sobolewska et al., 2017), or have no effects (Awad et al., 2009, Sobotik et al., 2021) on crypts depth. It should be noted that the interpretation of the obtained data was complicated by the fact that the two treated houses also exhibited significant differences in villi and crypts length. Nonetheless, the existence of an actual beneficial effect of the synbiotic treatment on intestinal morphology is supported by the overall agreement between the two treated houses compared to the control one, and by the general increase seen in the ratio between villi and crypts length.

The use of high-throughput sequencing provided useful insights into the composition of the caecal bacterial population. However, exactly defining a healthy intestinal microbiota is not an easy task, as it is influenced by a multitude of environmental and host-related factors, such as litter, housing, climate and the chickens' age, sex and breed (Kers et al., 2018). The overall bacterial diversity was rather high and was shown to increase with age, in agreement with previous studies (Videnska et al., 2014; Ocejo et al., 2019). A highly diverse bacterial community is indicative of good intestinal health, while a reduced heterogeneity could signal intestinal disease states (Ocejo et al., 2019; Madlala et al., 2021). The observed caecum composition was in agreement with what was expected in poultry, exhibiting a clear predominance of *Firmicutes*, and, in particular, of families belonging to the class *Clostridia*, such as *Lachnospiraceae*, *Methanobacteriaceae*, and *Ruminococcaceae* (Clavijo and Florèz, 2018; Such et al., 2021). *Firmicutes* are associated with butyrate production, while *Bacteroidetes*, which represent a small fraction of the caecal microbiota, are involved in the production of propionate. Their ratio is commonly accepted as an indicator of the efficiency of energy harvesting in both humans and animals (Zhu et al., 2019). Videnska et al. (2014) studied the development of the caecal microbiota in laying hens over the entire production cycle. They reported that the relative abundance of *Bacteroidetes* increased between the second and the sixth month while *Firmicutes* were predominant during the first month of age, leading to an even ratio between the two phyla in adult hens. Several studies also reported *Firmicutes* to be predominant in broiler chickens and young hens (Bjerrum et al., 2006; Nordentoft et al., 2011; Videnska et al., 2013), while members of

Bacteroidetes seem more abundant in older chickens (Callaway et al., 2009). While this shift has not been observed in the present study, with *Firmicutes* being by far the predominant phyla even at 40 weeks of age, it should be considered that the F/B ratio is heavily determined by the administered feed (Nordentoft et al., 2011) and that it has never been investigated before in broiler breeders, thus preventing comparisons with chickens sharing the same genetic features and producing conditions.

The treatment effect on bacterial composition was confirmed to be statistically significant and led to a differential abundance of 119 ASVs. Among the most impacted were members of the families *Lachnospiraceae* and of the genus *Helicobacter*, which were overrepresented in treated chickens, and of *Ruminococcaceae*, which in turn were underrepresented. More puzzlingly, members of *Gastranaerophilales* and *Clostridia* were found among both the most over and underrepresented ASVs in treated chickens. All these bacteria are common inhabitants of the caecal microbiome (Aruwa et al., 2021; Gilroy et al., 2021; Xiao et al., 2021), and their abundance was already proven to be modulated by several nutraceuticals. Díaz Carrasco et al. (2018) found that tannins administration increased the relative abundance of *Helicobacter* and, more importantly, of members of both *Lachnospiraceae* and *Ruminococcaceae* (and decreased other members of the two families), possibly shifting the short-chain fatty acids caecal profile towards butyrate production. Li et al. (2020) reported that the supplementation of fermented soybean meal in broilers led to an increased abundance of *Gastranaerophilales*, which in turn was positively correlated to an improved average daily gain and serum immunity.

Previous studies relying on high-throughput sequencing already investigated the effect of synbiotics with different compositions on chickens' intestinal microbiota, but, to the authors' knowledge, this is the first time this technique is carried out in broiler breeders, not allowing a comparison with chickens with similar genetic traits and raised under the same production system. Pineda-Quiroga et al. (2019) found that treating laying hens with a synbiotic product based on dry whey powder and *Pediococcus acidilactici* increased the caecal abundance of *Actinobacteria*, *Olsenella* spp., and *Lactobacillus crispatus*, among others. The double administration of a multi-species synbiotic, both by spray at the hatchery and in the feed throughout the broiler cycle, caused an increased abundance of *Actinobacteria* and *Lactobacillus* spp. as well, along with several members of *Clostridia*, and also led to a higher *Firmicutes* to *Bacteroidetes* ratio (Brugaletta et al., 2020). Another trial conducted in broiler chickens found that a synbiotic containing *Bacillus subtilis*, yeast, and inulin did not affect the caecal microbiota (Such et al., 2021). The diversity in the results obtained by these studies can be easily justified by the many variables at play (experimental design, synbiotic composition and dosage, productive type, breed, age at sampling, feed, and rearing conditions) and by the inherent complexity of the caecal ecosystem, which hosts the largest (and partially unculturable) bacterial population out of all intestinal tracts (Aruwa et al., 2021). On the other hand, this adds value to the herein reported data, which are among the first to provide a longitudinal perspective on the enteric microbiome of broiler breeders.

CONCLUSION

Based on the reported results, the synbiotic product PoultryStar® sol appears fully applicable to broiler breeders through intermittent drinking water administration. Histopathological and morphometrical findings support its beneficial effect on gut health, and higher survivability was also observed in treated chickens during the production phase. In addition, the synbiotic treatment had a modulating effect on several bacterial populations hosted in the caeca, whose actual impact will require further investigations to be fully elucidated.

DECLARATIONS

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Authors' contribution

Zoi Prentza contributed to the conceptualization, investigation, data curation, writing, review and editing of the manuscript. Francesco Castellone participated in investigation activities. Matteo Legnardi contributed to data analysis and visualization, writing, review and editing processes. Birgit Antlinger was involved in the conceptualization of the study. Maia Segura-Wang participated in investigation activities. Giorgos Kefalas was involved in the conceptualization process and in resource provision. Paschalis Fortomaris, Angeliki Argyriadou, Nikolaos Papaioannou, and Ioanna Stylianaki participated to data analysis and visualization. Giovanni Franzo participated to data curation, analysis, and visualization. Mattia Cecchinato and Vasileios G. Papatsiros supervised the project. Kostantinos Koutoulis was responsible for the conceptualization, resource provision, supervision, and project administration. All

authors checked and approved the final version of the manuscript for publishing in the present journal.

Competing interests

The funders were not involved in the study design, data collection, and analysis, nor in the writing of the manuscript.

Ethical considerations

All relevant ethical issues have been checked by all the authors.

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Identification of Adeno-associated Virus in Muscovy Ducks with Chronic Diarrhea

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ABSTRACT

Adeno-associated viruses (AAVs) are defective members of the genus *Dependoparvovirus*. Waterfowl parvoviruses, another member of the *Dependoparvovirus*, were found to be the closest relative of AAVs. This study was performed to identify the genetic changes that may occur to goose parvovirus (GPV) in one Muscovy duck flock that was observed for 12 weeks after the virus was isolated. Persistent watery diarrhea and wing deformity were the common signs. Cloacal swabs were collected from diseased ducks. Unexpectedly, the identified virus was an AAV. The closest strains were duck AAVs at the nucleotide level, identified in Australia and China. Meanwhile, only 52.3% of nucleotide identity was shared with the GPV strain, previously identified from this flock. Duck adenovirus (DAdV) could not be identified in the samples. This study is one of the first studies in which genetic changes of GPV were tracked. In addition, emerging duck AAV from GPV is suggested, which will be useful for future virus classification.

Keywords: Adeno-associated virus, Chronic diarrhea, Muscovy ducks

INTRODUCTION

Adeno-associated viruses (AAVs) are defective and considered promising therapeutic viral vectors because of their *in vivo* transduction ability with induction of mild immune response and with no evidence of toxicity (Bello et al., 2014; Samulski and Muzyczka 2014; Bennett et al., 2017). Due to their defectiveness, these viruses can not complete their replication cycle except with a helper virus that can be Adenovirus, Herpes virus, Varicella, Cytomegalovirus, or Bocavirus (Georg-Fries et al., 1984; Ni et al., 1994; Wang et al., 2017). In addition to helper viruses, carcinogens and genotoxic agents can render cells permissive to the replication of AAVs (Schlehofer et al., 1986; Yakinoglu et al., 1988; Berns 1990). Without any helper virus, latent infection is established (Berns 1990; Sun et al., 2010; Meier et al., 2020). Viral particles of AAVs consist of a small nonenveloped capsid of about 260 Å in diameter that is composed of three viral proteins: VP1, VP2, and VP3. Their genome is single-stranded DNA of about 4.7-kb with identical inverted terminal repeats at both ends of approximately 150 nucleotides that have genome replication and packaging signals (Bennett et al., 2017; Hildebrandt et al., 2020). Genus *Dependoparvovirus*, a member of the family Parvoviridae, comprises AAVs and the autonomous waterfowl parvoviruses (goose and Muscovy duck parvoviruses, Kailasan et al., 2015; Hildebrandt et al., 2020). Goose parvovirus (GPV) is the causative agent of Derzsy's disease (Derzsy, 1967; Kisary and Derzsy, 1974) that affects Muscovy ducks and geese and is characterized by growth retardation, feathering disorders, and is associated with high mortality rates (Tatár-kis et al., 2004; Glávits et al., 2005). A GPV-related group causes short beak and dwarfism syndrome, and the affected Muscovy ducks show feathering disorders, short beak, tarsus, strong growth retardation, and low morbidity rate (Palya et al., 2009). Muscovy duck parvovirus infects the ducks, causing weakness, locomotor problems, and recumbency (Poonia et al., 2006; Palya, 2020). Duck adeno-associated virus (DAAV) was first identified in clinical samples collected from Muscovy ducks with signs of adenovirus infection in China (Su et al., 2017). Another distantly

related DAAV was identified in fecal samples collected from wild Pacific black ducks in Australia (Vibin et al., 2020). The current study was conducted to re-identify goose parvovirus (GPV) in Muscovy ducks 12 weeks after the first isolation of the virus from these ducks and to determine the genetic changes that may occur due to chronic infection.

MATERIALS AND METHODS

Ethics approval

Samples were collected according to the Animal Care and Biosafety Committee of the Animal Health Research Institute (AHRI 121119).

Samples

Goose parvovirus strain HS1 (accession Number OL763424) was isolated from cloacal swabs obtained from a Muscovy duck flock that consisted of 4 weeks old 60 female and 10 male ducks in Behira governorate, Egypt, in May 2020. These ducks had no vaccination history and suffered from retarded growth, loss of feathers, recumbency, whitish watery diarrhea, and wing deformity. This flock was observed for a study period that extended for 12 weeks post-isolation of GPV. At the end of the study period, cloacal swabs were collected from 15 ducks with chronic diarrhea and wing deformity to determine the possibility of the persistence of GPV infection. Swabs were pooled, suspended in phosphate buffered saline, and centrifuged (Germany) at 8000 × g for 15 minutes. The supernatant was filtered through a 0.22 µm filter and kept in -80°C until the detection of viral DNA by Polymerase chain reaction (PCR).

Viral DNA detection by the polymerase chain reaction

To avoid any genetic mutation that may occur during viral isolation because of viral adaptation to duck embryo or tissue culture, viral DNA was extracted directly from the samples without viral isolation. DNA extraction was performed using QIAamp Mini Elute Virus Kit (Qiagen, Germany) following the manufacturer's instructions. Extracted DNA was amplified using a buffer mix (Emerald Amp Max, Takara, Japan) and primers listed in Table 1. Primers directed to partially amplify the VP1 gene of GPV were used based on the previous history of the infection, whereas DAAV was detected unintentionally with the same primers but with different sizes of amplified sequences. Due to the identification of DAAV in the samples, another set of primers was used to detect duck adenovirus (DAdV). The PCR was performed using Biometra T3000 thermocycler (Biometra, Germany). Electrophoresis was done through 1.5% agarose gel and a 100 bp DNA marker (Thermo Scientific, USA).

Table 1. Primers used in the study

Virus	Primers	References
Goose parvovirus Duck adeno-associated virus	F (5'-CCTGGCTATAAGTATCTTGG-3') R (5'-GTAGATGTGGTTGTTGTAGC-3')	Poonia et al., 2006
Duck adenovirus	F (5'-CACTCACGGGAACTG-3') R (5'-GGGCACCACAAACG-3')	Zhang et al., 2016

DNA sequencing

A single 609 bp DNA band was purified using a QIAquick gel extraction kit (Qiagen, USA) and sequenced in both directions with the same primers indicated in Table 1. The sequencing reaction was done using the Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer, USA) in ABI automated sequencer (Applied Biosystems 3500xl genetic analyzer, USA).

Phylogenetic analysis

The obtained partial sequences of VP1 were subjected to a basic local alignment search tool (BLAST) within GenBank to determine the closely related strains. Nucleotide sequences and their deduced amino acids were aligned using CLUSTALW with 1000 bootstrap replications in BioEdit software (version 7.2.5). The maximum composite likelihood method with 1000 bootstrap replications of MEGA11 software was used to determine pairwise distance and to construct a neighbor-joining (NJ) tree (Tamura et al., 2021).

RESULTS AND DISCUSSION

Adeno-associated viruses share the same genome organization as waterfowl parvoviruses (Zadori et al., 1995). Moreover, at the level of amino acid sequence, waterfowl parvoviruses were found to be the closest relative of adeno-associated virus 2. Thus, GPV was reclassified with AAV under the genus

Dependoparvovirus (Brown et al., 1995; Zadori et al., 1995).

Goose parvovirus was isolated and identified from a Muscovy duck flock with retarded growth, feather disorders, diarrhea, and wing deformity. During this study, this flock was observed for 12 weeks after GPV was isolated to determine the persistent possibility of clinical signs associated with GPV infection. During this period mortality rate was 30%, and the common signs were chronic persistent watery diarrhea and wing deformity, which were reported in about 80% of the affected flock. Therefore, cloacal swabs were collected as pooled samples from ducks that suffered from these symptoms to re-identify GPV in this flock. The identified virus was AAV, not GPV. Both sites of the primers used in the detection of GPV (Table 1) were similar to the analogous sites at the identified DAAV (with only two nucleotide differences in forwarding primer), which enabled its unintended detection. Based on the partially DNA sequenced VP1, the identified virus designated HSCH (accession number. ON166703) shared only 52.3% and 49.1% nucleotide and amino acid identities with the GPV (HS1), respectively, which was previously isolated from this flock (Table 2). This DNA sequence which includes the N terminus of VP1, 2, and 3, was found to be 15 bases longer than that of GPV due to many nucleotides' insertions.

The closest strain was PBDAAV/PBD12, which was identified from wild ducks in Australia with 87.4% and 97.4% nucleotide and amino acid identities, respectively (Vibin et al., 2020). Phylogenetic analysis revealed separate clustering of the identified strain within avian *Dependoparvoviruses*, including autonomous waterfowl parvoviruses, DAAVs, and avian AAVs (AAAVs), which all shared the same ancestor (Figure 1). In addition, the identified strain possessed 18 unique amino acid substitutions, most of which were located at the N terminus of VP2 and 3 (Figure 2). A nucleotide identity of 82.7% was found with DAAV (MHH-05-2015) that was also identified in Muscovy duck in China (Su et al., 2017). DAAV (MHH-05-2015) virus was found to be dependent on DAdV infection. Therefore, an attempt was made to detect co-infection with DAdV. The DAdV could not be identified in the samples. Adeno-associated viruses have dependent replication nature as they need another helper virus, carcinogen, or genotoxic agent to complete their replication cycle (Schlehofer et al., 1986; Yakinoglu et al., 1988; Ni et al., 1994; Wang et al., 2017). Accordingly, it can be suggested that this flock may be affected by a helper agent other than DAdV. Another possible explanation for the absence of DAdV from the sample is the emergence of the identified duck AAV from GPV strain HS1, which was previously identified from the same flock. Hildebrandt et al. (2020) have done phylogeny reconstruction of endogenous viral elements of *Dependoparvoviruses* (viral genetic remnants integrated into host genomes for millions of years) and suggested the autonomous highly pathogenic exogenous *Dependoparvovirus* ancestors of these elements that co-evolved with waterfowl birds. This finding and the persistence of diarrhea since GPV infection support the suggestion of the emergence of DAdV from GPV. This was also supported by Kailasan et al., 2015 who reported that parvoviruses had high evolution rates similar to RNA viruses, and cell passages with very small numbers could induce the selection of new natural mutants.

Table 2. Nucleotide and amino acid identities with other goose parvoviruses and duck adeno-associated viruses identified in Muscovy ducks

	Amino acids identities									
Strains	1	2	3	4	5	6	7	8	9	10
1-M15		98.5	96.4	97.4	79.3	98	49.9	50.8	49.9	50.8
2- JS1603	98.6		95.9	96.9	79.9	97.4	49.9	50.8	49.9	50.8
3- LH	95.4	95.6		99	79.3	95.3	50.8	51.6	51.6	52.4
4- B	96.7	97.1	97.6		80.5	96.4	51.6	52.4	51.6	53.2
5- FM	77.5	78.3	78	78.6		78.7	45.7	49.1	49.1	49.1
6- HS1	97.6	97.4	96	96.9	77.8		49.1	49.1	48.2	49.1
7- HSCH	51.3	52	55	52.6	53.4	52.3		85.3	84.8	85.9
8- PBDAAV/PBD12	54.1	54.7	56.5	54.4	59	54	87.4		97.4	87.1
9- DAAV/G002-20	54	54.6	56.4	55	60.2	54.4	86.5	97.9		85.9
10- MHH-05	50.9	51.7	53.7	52.2	56.9	50.6	82.7	80.4	80.4	
	Nucleotides identities									

The strain that was identified during this study is highlighted

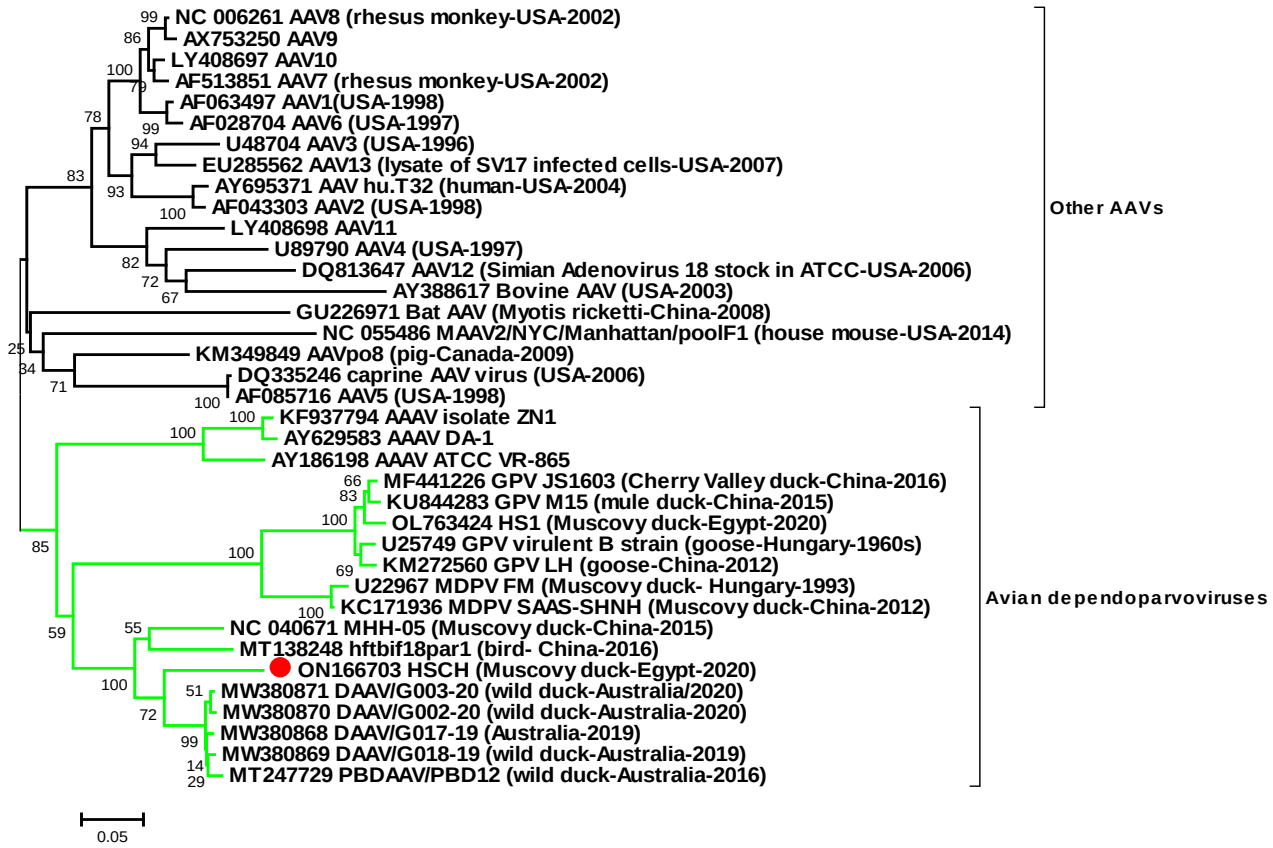

Figure 1. Neighbor-joining tree indicates clustering of the identified strain separately within avian *Dependoparvoviruses* (autonomous waterfowl parvoviruses and AAAVs) identified in Muscovy ducks. This tree shows a common ancestor with avian *Dependoparvoviruses*. Green branches indicate avian *Dependoparvoviruses* while black branches indicate AAVs other than avian origin. The red circle indicates the identified strain.

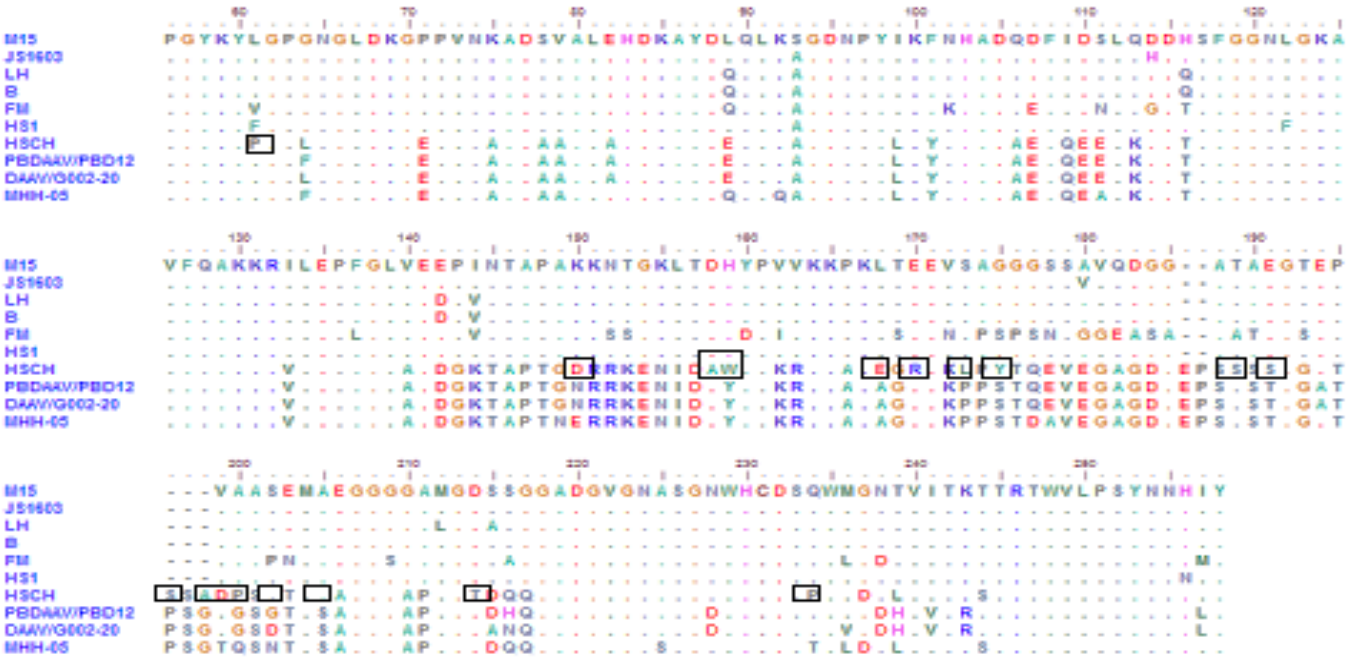

Figure 2. Amino acids substitutions with autonomous *Dependoparvoviruses* and closely related duck adeno-associated viruses

This study is one of the first identification of DAAV from Muscovy ducks in Egypt. Moreover, it indicates the possibility of emerging DAAV from GPV. Further study should be done experimentally to confirm the possibility of emerging DAAV from GPV to understand the pattern of viral evolution better.

DECLARATIONS

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Authors' contribution

Hamdi Mohamed Sallam designed the study, collected the samples, carried out the molecular genetic study, and wrote the manuscript. Ali Mahmoud Zanaty performed DNA sequencing and revised the manuscript. All authors checked and approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors declare that they have no competing interests.

Ethical consideration

All ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all authors.

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Increasing the Quality of Blood Tofu in an Industrial Slaughterhouse of Thailand

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ABSTRACT

Blood tofu, or cooked duck blood curd, is a Chinese delicacy in East Asia. Its quality and shelf-life are low due to microorganism contamination during production. Therefore, the present study was performed to investigate the role of sodium diacetate (SD), sodium chloride (NaCl), and polyethylene glycol 400 (PEG) combinations in increasing the quality of blood tofu. A total of 45 cooked duck blood curd samples were randomly divided into 3 groups with 3 replicates per group. The first two groups were used to investigate the effect of SD, NaCl, and PEG combinations on microbiological and physical analyses for non-inoculated samples. Another group was used to determine the effect of antimicrobial combinations on *Lactobacillus plantarum*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus* in inoculated samples that were inoculated with these bacteria. All groups were treated with control-sterilized water, 0.15% SD (w/v) + 1.25% NaCl (w/v), 0.30% SD (w/v) + 1.25% NaCl (w/v), 0.15% SD (w/v) + 0.15% PEG (w/v), and 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). The results indicated that soaking cooked duck blood curd samples in antimicrobial agent combinations could reduce mesophile and psychrophile bacteria counts in non-inoculated samples. Additionally, 0.15% SD + 1.25% NaCl + 0.15% PEG combination had a higher reduction in mesophile and psychrophile counts, compared to soaking the samples in 0.30% SD + 1.25% NaCl, 0.15% SD + 1.25% NaCl and 0.15% SD + 0.15% PEG combinations. Similarly, this combination showed a significant decrease in lactic acid bacteria, *Pseudomonas*, *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* counts in inoculated samples. Furthermore, soaking the samples in 0.15% SD + 1.25% NaCl + 0.15% PEG combination did not negatively affect the samples' physical quality. Soaking the samples in 0.15% SD + 1.25% NaCl + 0.15% PEG combination inhibited the growth of mesophile, psychrophile, and *Pseudomonas* in non-inoculated samples after storage for 10, 6, 10, and 8 days in a slaughter warehouse at 7℃, respectively, and extended shelf-life of samples for 16 days. Regarding physical quality changes, this treatment delayed the reduction of pH, hue, hardness, and chewiness of the samples after storage for 10, 8, 12, and 10 days, respectively. Thus, SD, NaCl, and PEG combination had a high preservative potential for cooked duck blood curd used in industrial slaughterhouses.

Keywords: Blood curd, Duck, Organic acid salt, Polyethylene glycol, Quality changes

INTRODUCTION

Blood is the first by-product collected during the meat-producing system in industrial slaughter. Due to its valuable nutritional value and functional properties, blood can be considered a beneficial raw material in the feed and food industries (Toldrá et al., 2016). Blood comprises 75-82% water and 17-18% protein, like oxyhemoglobin and methemoglobin, which exist in red blood cells (Leoci, 2014). Nevertheless, blood can be contaminated by pathogens, such as lactic acid bacteria (LAB), *Pseudomonas*, *Salmonella*, *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*) during the slaughter process through contact with animals' skin, stomach, and intestines. These microbial can be brought into the blood and grow quickly (Dàvila et al., 2006).

Cooked blood curd or blood tofu is one of the famous Chinese foods in East Asia. Normally, it is served with noodles, congee, and soup. The cooked blood curd is prepared from fresh blood coagulating by setting fresh blood in a container. Then, it is cooked in heated water. After the product cooling, it is packed in a plastic bag containing water (Wang and Lin, 1994). The short shelf-life of the product may be due to the survival of contaminated microorganisms in the production process (Wang et al., 2010).

Sodium diacetate (SD) is derived from acetic acid. It acts as a bactericidal agent by expanding the lag phase of spoilage and pathogenic microbial and extending the shelf life of meat and meat products (FDA, 2016). Sodium diacetate is approved by the United States Food and Drug Administration and is generally recognized as a safe ingredient in food products (FDA, 2000). However, the maximum recommended level for SD is up to 0.25% due to off-flavor detection (FDA, 2016). At 0.20% concentration of SD, it has an unfavorable effect on the odor and taste of food products (Stekelenburg and Kant-Muermans, 2001). For sodium chloride (NaCl), a high salinity induces water efflux which is counterpoised by a rise of cooperative solutes (Krämer, 2010). However, the use of high concentrations of salt can adversely affect the consumers' perception of overall palatability (Omotoyinbo and Omotoyinbo, 2016). Polyethylene glycol (PEG) 400 is a surfactant for food additive Generally Recognized as Safe (GRAS, 21 CFR 178.3750, FDA, 2022). Increasing the level of PEG to 20% (w/v) could effectively inhibit the growth of various pathogenic bacteria, including *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Chirife et al., 1983; Holcapkova et al., 2018).

In a previous study by Tangwatcharin and Teemeesuk (2019), the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SD and NaCl to inhibit the growth of *E. coli*, *S. aureus*, *S. typhimurium*, and *Pseudomonas fluorescens* (*P. fluorescens*) in medium broth were determined. The fractional inhibitory concentration index (FBCI) indicated that the utilization of 0.15% (w/v) SD + 1.25% (w/v) NaCl resulted in improved inhibition of these pathogenic bacteria. Moreover, this study achieved *in vitro* antimicrobial efficacy of this combination to control the growth of pathogenic bacteria. Additionally, the 0.16%(w/v) SD + 0.16%(w/v) PEG combination appeared to have a significant inhibitory effect on *S. aureus* and *E. coli* and extended the shelf life of fresh ground pork for 12 and 8 days of storage at 5℃ and 15℃, respectively (Tangwatcharin et al., 2018). With this in mind, the current study aimed to evaluate the effects of SD, NaCl, and PEG combinations on microbiological and physical qualities of cooked duck blood curd and their application in slaughter warehouses.

MATERIALS AND METHODS

Bacterial strains and inocula

Lactiplantibacillus plantarum (*L. plantarum*) TISIR543, *P. fluorescens* DMST20076, *S. Typhimurium* DMST22842, *E. coli* DMST4212, and *S. aureus* DMST4745 were obtained from the culture collection at Department of Medical Sciences, Ministry of Public Health, Thailand. Each strain was cross-linked on Mueller Hinton agar (MHA, Merck, Germany) and incubated at 35℃ for 18 hours. These cultures were prepared by inoculating 10 ml of 0.90% (w/v) sodium chloride (NaCl, normal saline) with 2-3 colonies taken from MHA. Inocula were prepared by diluting in 10 ml of normal saline to 10^8 CFU/ml (McFarland standard of 0.5). As required, these suspensions were further diluted with 99 ml of normal saline (1:100 dilution). The initial concentrations were adopted at approximately 1×10^6 CFU/ml (Tangwatcharin et al., 2018).

Experiment design

Cooked duck blood curd samples (*Cherry valley* crossbred ducks at 47 days) were collected in three batches on different days in a cooked duck blood curd line in an industrial slaughterhouse in East Thailand. All samples were weighed 500 g/sample, packed in a polyethylene bag filled with slaughterhouse water consumption, kept in a polystyrene box containing ice, and transported to a laboratory room within 3 hours for further analysis (Tangwatcharin and Teemeesuk, 2019). Then, 45 samples were divided into 3 groups. Two groups were used to investigate the effect of SD, NaCl, and PEG combinations on microbiological (one group) and physical analyses (one group), for which the samples were not inoculated with the inocula following Tangwatcharin et al. (2019a). Another group was used to determine the effect of antimicrobial combinations on *L. plantarum*, *P. fluorescens*, *S. typhimurium*, *E. coli*, and *S. aureus*. The cooked duck blood curd samples were inoculated with all 5 bacteria suspensions. The samples were individually soaked in 300 ml of each bacterial inoculum (10^6 CFU/ml) for 20 minutes and dried in laminar airflow. The initial count of each bacterium was 10^4 CFU/g. After that, all samples were weighed. For all groups, the cooked duck blood curd samples were randomly divided into 5 treatments and soaked in 300 ml of antimicrobial, including T1 for control in which sterilized water was used, T2 for 0.15% SD (w/v) + 1.25% NaCl (w/v), T3 for 0.30% SD (w/v) + 1.25% NaCl (w/v), T4 for 0.15% SD (w/v) + 0.15% PEG (w/v), and T5 for 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). Each group was packed in the polyethylene bag, stored at 7℃ for a day, and weighed before analysis. Then, microbiological and physical qualities were estimated. This experiment was replicated three times.

Later, changes in microbiological and physical qualities of cooked duck blood curd soaked in SD, NaCl, and PEG combination during cold storage were determined. Non-inoculated samples were prepared according to the method reported by Tangwatcharin et al. (2018). Samples were randomly divided into two treatments as soaking in slaughterhouse water consumption (control) and 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). The quality of slaughterhouse water consumption was checked monthly, and the lower limit of drinking water standard no. 257-2549 for the food industry of Thailand was considered (Thai Industrial Standard Institute, 2006). Samples were stored in the slaughter warehouse at 7℃ for 18 days, and collected on days 1, 2, 4, 6, 8, 10, 12, 14, 16, and 18. The collected samples were transported and analyzed within 3 hours.

Microbiological analyses

Twenty-five g of the sample was diluted in 225 ml of 0.85% NaCl (w/v, saline solution) and homogenized in a stomacher bag mixer (Interscience, France). The homogenate was serially diluted (1:10) with saline solution. For non-inoculated samples, each dilution was grown in plate count agar (PCA, Merck, Germany). The samples were then incubated at 35 ± 2℃ for 24-48 hours for mesophile count (BAM, 2001a) and at 7 ± 2℃ for 10 days for psychrophile count (ISO, 2001). Furthermore, yeast and mold counts were analyzed by plating in potato dextrose agar (PDA, Merck, Germany) and then incubated at 25 ± 2℃ for 5 days (BAM, 2001b).

For inoculated samples, the following media and incubated conditions were employed. For *Pseudomonas* spp. count, *Pseudomonas* CFC agar (Oxoid, United Kingdom) was used with incubation of samples at 25 ± 2℃ for 24-48 hours (Rajmohan et al., 2002). For *Samonella* spp. count, xylose lysine deoxycholate (Merck, Germany) was utilized with incubation of samples at 37 ± 2℃ for 24-48 hours (Van der Zee, 2003). *Escherichia coli* count was measured using violet red bile agar (Merck, Germany) and incubation of samples at 37 ± 2℃ for 24-48 hours (Tangwatcharin et al., 2018). *Staphylococcus aureus* count was gauged using Baird Parker agar (Merck, Germany) by adding 5% (v/v) egg-yolk tellurite emulsion 20% (Merck, Germany) and then incubation of samples at 37 ± 2℃ for 24-48 hours (BAM, 2001c). The LAB was counted by de Man, Rogosa, and Sharpe agar and anaerobic incubation of samples at 30℃ for 24-48 hours (Bover-Cid and Holzapfel, 1999). Finally, oxidase, aerobics, and ability to ferment glucose for *Pseudomonas* spp., triple sugar iron agar test, lysine iron agar test for *Salmonella* spp., Indole, Methyl Red, Voges Proskauer and Citrate (IMViC) tests for *E. coli*, and coagulase test for *S. aureus* were performed to confirm the findings. The results were transformed to log cfu/gram of sample (log cfu/g).

Mesophile, psychrophile, yeast and mold, LAB, *Pseudomonas* spp., *S. aureus* counts were analyzed using previous methods to indicate the changes in the microbiological quality of cooked duck blood curd soaked in SD, NaCl, and PEG combination during cold storage in slaughter warehouse. Additionally, *Bacillus* spp. counts were determined using the methods proposed by Turner et al. (1996). Later, the enrichment and the most probable number (MPN) method was used for *Salmonella* (ISO, 2017), *Listeria monocytogenes* (BAM, 2017), *E. coli* (BAM, 2020), and *Clostridium* spp. (Turchi et al., 2016; Tangwatcharin et al., 2019a).

pH

For pH measurement, the non-inoculated sample was directly determined at five different locations using a portable pH meter (Mettler Toledo SevenGo SG2, Mettler Toledo, Switzerland).

Weight loss

Cooked duck blood curd samples were first weighed before being soaked in sterilized water or antimicrobial and second weighed after cold storage. The sample weights were applied to compute the weight loss (%)**.**

Color

The color was measured on the cut surface of non-inoculated samples. The International Commission on Illumination (CIE) L* (lightness), CIE a* (redness), and CIE b* (yellowness) values were estimated by using a HunterLab MiniScan EZ 4000L (Hunter Associates Laboratory, USA). For each sample, the means of the readings were obtained at five locations. Hue angle (h°) was calculated according to the equation:

$$\text{Hue angle (h°)} = \text{arctg (CIE b*/ CIE a*)}$$

Texture profile analysis

Three pieces of each sample were cut into cubes (15×15×15 mm). Texture profile analysis (TPA) was estimated using an Instron universal testing (model 3344, USA) by a cylindrical aluminum probe, 55 mm inner diameter at room temperature (25℃). Texture profile analysis textural variables were estimated with crosshead

speed 1 mm/sec, holding time 1 second, working distance 40% strain (Bourne, 2002). The collection and processing of data were performed by the Blue-hill 2 software (Instron Engineering Corporation, USA). Among the textural parameters analyzed during a TPA test, only hardness, cohesiveness, gumminess, springiness, and chewiness were estimated for the force-time curves generated for each sample.

Statistical analyses

Each experiment was replicated three times. The SPSS (version 28) was used for the statistical analyses. The data were analyzed by one-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine the mean comparison ($p < 0.05$). Pearson's correlation coefficients were carried out to determine the relationship among variables.

RESULTS AND DISCUSSION

Qualities of cooked duck blood curd

Soaking the samples in antimicrobials significantly affected mesophiles and psychrophiles in non-inoculated cooked duck blood curd ($p < 0.05$, Table 1). The samples soaked in 0.15% SD + 1.25% NaCl + 0.15% PEG were the lowest these microbial counts ($p < 0.05$). Moreover, the soaking samples in 0.15% SD + 1.25% NaCl + 0.15% PEG combination exposed higher reduction of mesophile and psychrophile counts than those of 0.30% SD + 1.25% NaCl, 0.15% SD + 1.25% NaCl, and 0.15% SD + 0.15% PEG ($p < 0.05$). The reduction of spoilage microorganism count were included 1.82 ± 0.07, 1.63 ± 0.11, 1.59 ± 0.09, and 1.47 ± 0.13 log cfu/g, respectively for mesophile and 1.40 ± 0.12, 1.17 ± 0.09, 1.08 ± 0.08, and 0.96 ± 0.13 log cfu/g (means ± standard deviation), respectively, for psychrophile when compared with control ($p < 0.05$). Moreover, yeast and mold were not detected in all samples (<1 log cfu/g).

Similarly, soaking the samples in antimicrobials reduced LAB, *Pseudomonas* spp. *Salmonella* spp., *E. coli*, and *S. aureus* counts in inoculated cooked duck blood curd ($p < 0.05$, Table 1). The samples soaked in 0.15% SD + 1.25% NaCl + 0.15% PEG combinations showed the lowest counts of the investigated bacteria ($p < 0.05$). In a previous study, the FBCI of 0.15% SD + 1.25% NaCl was 0.25 against *E. coli* and 0.62 against *S. Typhimurium* and *P. fluorescens* (Tangwatcharin and Teemeesuk, 2019). Sodium diacetate is a weak organic acid salt that effectively inhibits most tested bacteria and connects with the outer surface of bacterial cells, resulting in the disorder of cell membrane integrity and leakage of the intracellular lysate and dissolution of the cytoplasmic membrane (Tangwatcharin et al., 2018). The physical stress in the cellular structure of *E. coli* and *Salmonella* causes cell damage, or shrinkage due to a low salinity, which leads to an immediate influx of small solutes (Hajmeer et al., 2006; Krämer, 2010; Omotoyinbo and Omotoyinbo, 2016). Adding 0.16% SD + 0.16% PEG was a potential antimicrobial for reducing *E. coli* and *S. aureus* in fresh ground pork (Tangwatcharin et al., 2018). This could be due to the synergistic antibacterial efficacy of PEG. Lipopolysaccharide (LPS) in bacterial cells, as well as the cell phospholipid, is a nonpolar surface, especially in Gram-negative bacteria (Rosen, 2004). These nonpolars allow the non-ionic PEG surfactant linked by SD + NaCl combination to associate with the outer surface of the target bacterial cells, leading to disorders in cell membrane integrity and finally the intracellular lysate leakage and cytoplasmic membrane dissolution (Tangwatcharin et al., 2018).

Table 2 shows the color measured by the instrument. There was no significant difference between samples soaked in water (control) and 0.15% SD + 1.25% NaCl ($p > 0.05$). However, a high concentration of SD (0.30% SD) had a significantly negative impact, especially on the CIE a* and hue ($p < 0.05$). The color of blood is due to the demeanor of hemoglobin molecules in the red blood cells (Leoci, 2014). After cooking, this globin is denatured and reduced heme. Hemochromogen and hemichromogen are formed from oxyhemoglobin and methemoglobin, which are dull red and brown pigments, respectively. However, reductions of hemichromogen appear after using reducing agents, such as SD. The porphyrin ring may be opened, forming a green verdohem. In the intense condition, the iron will be lost from the porphyrin, split from the protein moiety, and open out, forming the chair of pyrroles characterizing colorless bile pigments (Toldrá et al., 2016). In accordance with the present study, samples soaked in 0.30% SD combination with NaCl exhibited higher CIE a* and lower hue than those in other antimicrobials and control ($p < 0.05$). There were significant negative correlations between CIE a* and hue ($r = -0.861$, $p < 0.05$). Due to hemichromogen reduction, abiding red hemochromogen in cooked blood curd was more apparent. Nevertheless, the pH values, weight loss, CIE b*, and texture analysis profiles were not affected by soaking in antimicrobials ($p > 0.05$).

Table 1. Effect of sodium diacetate, sodium chloride, and polyethylene glycol in combinations on bacteria on non-inoculated and inoculated cooked duck blood curds

Bacteria	T1	T2	T3	T4	T5	p-values
Non-inoculated cooked duck blood curd (log cfu/g)						
Mesophile	3.62 ± 0.09^{a}	2.03 ± 0.13^{b}	1.99 ± 0.14^{bc}	2.15 ± 0.10^{b}	1.80 ± 0.05^{c}	< 0.05
Psychrophile	2.74 ± 0.12^{a}	1.66 ± 0.10^{b}	1.57 ± 0.08^{b}	1.78 ± 0.16^{b}	1.34 ± 0.07^{c}	< 0.05
Yeast and Mold	<1	<1	<1	<1	<1	
Inoculated cooked duck blood curd (log cfu/g)						
Pseudomonas spp.	3.91 ± 0.07^{a}	2.89 ± 0.10^{bc}	2.84 ± 0.16^{c}	3.11 ± 0.12^{b}	2.54 ± 0.15^{d}	< 0.05
Salmonella spp.	4.15 ± 0.04^{a}	2.64 ± 0.18^{c}	2.58 ± 0.09^{c}	3.15 ± 0.13^{b}	2.32 ± 0.06^{d}	< 0.05
Escherichia coli	4.16 ± 0.05^{a}	2.54 ± 0.14^{c}	2.47 ± 0.10^{c}	3.04 ± 0.08^{b}	2.27 ± 0.12^{d}	< 0.05
Staphylococcus aureus	4.09 ± 0.13^{a}	2.85 ± 0.08^{b}	2.80 ± 0.10^{b}	3.09 ± 0.14^{b}	2.51 ± 0.03^{c}	< 0.05
Lactic acid bacteria	4.02 ± 0.11^{a}	2.99 ± 0.05^{c}	2.91 ± 0.07^{c}	3.17 ± 0.06^{b}	2.71 ± 0.21^{c}	< 0.05

T1: Control, T2: 0.15% SD (w/v) + 1.25% NaCl (w/v), T3: 0.30% SD (w/v) + 1.25% NaCl (w/v), T4: 0.15% SD (w/v) + 0.15% PEG (w/v), T5: 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). Values are given as means ± standard deviation from triplicate determinations. ^{a,b,c,d} Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Table 2. Effect of sodium diacetate, sodium chloride, and polyethylene glycol combinations on the physical quality of non-inoculated cooked duck blood curd.

Parameters	T1	T2	T3	T4	T5	p-values
pH	7.54 ± 0.19	7.55 ± 0.15	7.34 ± 0.41	7.52 ± 0.24	7.57 ± 0.21	0.7832
Weight loss (%)	2.17 ±0.24	2.35 ± 0.31	2.51 ± 0.22	2.31 ± 0.29	2.29 ± 0.18	0.0825
CIE L*	32.98 ± 0.68	32.76 ± 0.39	32.21 ± 0.15	32.67 ± 0.41	32.70 ± 0.25	0.4492
CIE a*	11.12 ± 0.33^{b}	11.57 ± 0.37^{b}	12.55 ± 0.23^{a}	11.52 ± 0.24^{b}	11.50 ± 0.37^{b}	0.0001
CIE b*	20.65 ± 0.68	20.52 ± 0.61	21.24 ± 0.52	20.60 ± 0.22	20.55 ± 0.43	0.3061
Hue	61.70 ± 0.43^{a}	60.58 ± 0.65^{b}	59.42 ± 0.47^{c}	60.64 ± 0.31^{b}	60.87 ± 0.51^{ab}	0.0030
Hardness (N)	2.28 ± 0.29	2.60 ± 0.19	2.80 ± 0.22	2.52 ± 0.48	2.47 ± 0.31	0.7345
Cohesiveness (ratio)	0.60 ± 0.02	0.61 ± 0.03	0.58 ± 0.02	0.61 ± 0.03	0.60 ± 0.02	0.2826
Gumminess (N)	1.43 ± 0.16	1.56 ± 0.17	1.63 ± 0.12	1.58 ± 0.28	1.50 ± 0.15	0.4958
Springiness (ratio)	0.89 ± 0.01	0.86 ± 0.01	0.86 ± 0.01	0.86 ± 0.02	0.86 ± 0.01	0.3660
Chewiness (N)	1.29 ± 0.14	1.41 ± 0.15	1.54 ± 0.20	1.46 ± 0.21	1.32 ± 0.18	0.7212

T1: Control, T2: 0.15% SD (w/v) + 1.25% NaCl (w/v), T3: 0.30% SD (w/v) + 1.25% NaCl (w/v), T4: 0.15% SD (w/v) + 0.15% PEG (w/v), T5: 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). CIE L*: lightness, CIE a*: redness, CIE b*: yellowness, N: Newton unit. Values are given as means ± standard deviation from triplicate determinations. ^{a,b,c} Different letters within the same row indicate significant differences ($p < 0.05$).

Shelf life of cooked duck blood curd

For storage in the warehouse at 7°C, soaking the samples in 0.15% SD + 1.25% NaCl + 0.15% PEG could control the growth of mesophile and psychrophile in samples stored for 10 and 6 days, respectively, compared to the control before storage ($p < 0.05$). Additionally, it could extend the shelf-life of the sample to 16 days (Figure 1A). These microbial are a considerable indicator of cooked food quality and shelf-life in cold storage (Ercolini et al., 2009). Mesophile count was the lower limit of Thailand's food and container microbiology standard (Department of Medical Sciences, 2017), which was not higher than 6 log cfu/g for cooked food and stored at cold temperature. Soaking the samples in SD, NaCl, and PEG combination could control the growth of *Pseudomonas* spp. and LAB in samples stored for 8 and 10 days, respectively (Figure 1B). Normally, their contaminations in blood products were found during the bleeding process. Due to these bacteria growth at low temperatures, they restricted the shelf-life of blood products (Dàvila et al., 2006). In the current study, all samples found <1 log cfu/g for yeast and mold, *S. aureus* and *Bacillus* spp., and < 3 MPN/g for coliforms, *E. coli*, *L.*

monocytogenus, and *Salmonella* spp. and *Clostridium* spp. throughout 18-day storage.

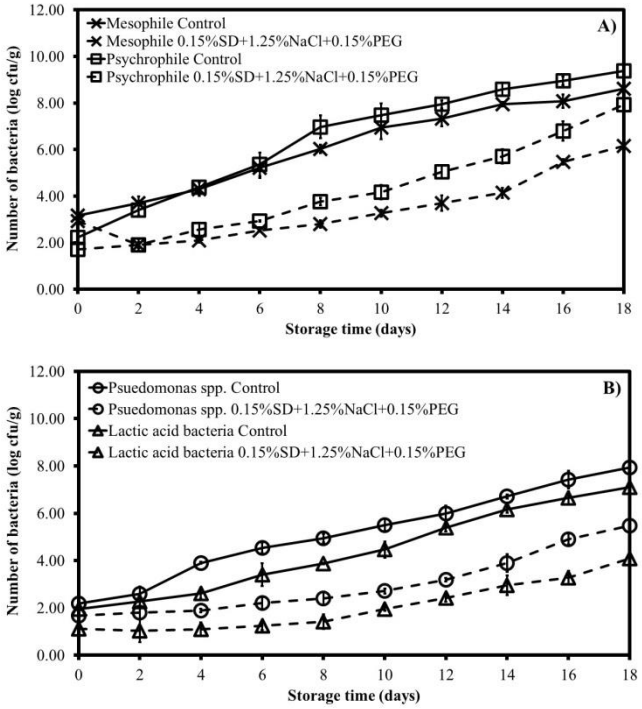

Figure 1. Effect of sodium diacetate (SD), sodium chloride (NaCl), and polyethylene glycol (PEG) combination on number of mesophile and psychrophile **(A)** and lactic acid bacteria and *Pseudomonas* spp. **(B)** of cooked duck blood curd stored at 7°C for 18 days in a slaughter warehouse.

The changes in physical quality of all samples stored in a warehouse at 7°C are demonstrated in Figure 2. Soaking the samples in an antimicrobial combination restrained the decrease of sample pH during storage for 14 days, then the pH decreased gradually throughout storage time. In contrast, the pH of control samples continuously reduced with an increase in storage time ($p < 0.05$, Figure 2A). The reason should ascribe to SD, NaCl, and PEG combination against LAB growth. Lactic acid bacteria generate lactic acid as a main metabolic end-product of carbohydrate fermentations, and then this increase in lactic acid is accompanied by a decrease in pH (Tangwatcharin et al., 2019b). In the present study, LAB count had a negative correlation with pH value ($r = -0.847$, $p < 0.05$).

On the contrary, the samples soaked in an antimicrobial combination delayed the increase of weight loss during storage for 10 days. After that, the weight loss increased with increasing storage time although the weight loss of control samples increased throughout storage time ($p < 0.05$, Figure 2B). Álvarez et al. (2009) reported that hemoglobin solubility varied in the 75-95% range, with the lowest value corresponding to pH 5.0 due to the protein precipitation. The solubility of duck blood powder decreased with decreasing pH values between 5 and 8 (Sorapukdee and Narunatsopanon, 2017). For NaCl soaking, the refolding of the protein was hampered by the presence of ions. An increase in levels of Cl^- ions causes a lower pH value, and proteins form progressively more stable, hydrophobic, and molten globular forms (Kristinsson and Hultin, 2004). The solubility in water influences other functional properties, especially the formation and stability of gels, resulting in changes in weight loss and texture characteristics during storage time (Álvarez et al., 2009; Sorapukdee and Narunatsopanon, 2017). In the present study, there was a significant negative correlation between pH and weight loss ($r = -0.798$, $p < 0.05$).

Regarding the color, the CIE a* and hue of all samples increased and decreased, respectively, with increasing storage time ($p < 0.05$, Figures 2C and 2D). However, soaking the samples in an antimicrobial combination decelerated the change of the hue of samples during the 8-day storage. Due to the microbial growth, they reduced hemichromogen and formed green choleglobin (Toldrá et al., 2016). Thus, cooked blood curd was a lighter shade of red with available red hemochromogen. In this study, mesophile and LAB counts were negatively correlated with hue value ($r = -0.839$ and -0.831, respectively, $p < 0.05$).

The changes in textural characteristics showed that the samples soaked in an antimicrobial combination delayed the increase of hardness and chewiness during storage for 10 and 12 days, respectively. The hardness increased faster throughout storage time. For the control sample, the hardness and chewiness of samples increased with increasing storage time ($p < 0.05$, Figures 3A and 3B). Due to the efficiency of SD, NaCl, and PEG combination against microbial, pH value and weight loss of the sample were reduced, and then they affected the decrease of hardness and chewiness. Mesophile, psychrophile, LAB counts and weight loss had a negative correlation with hardness ($r = -0.735$, -0.816, -0.817 and -0.861, respectively, $p < 0.05$) and with chewiness ($r = -0.722$, -07.95, -0.805 and -0.726, respectively, $p < 0.05$). Additionally, pH value had positive correlations with hardness and chewiness ($r = 0.874$ and 0.860, respectively, $p < 0.05$). Considering other textural characteristic, there were no significant differences in

cohesiveness, gumminess, and springiness of all samples ($p > 0.05$).

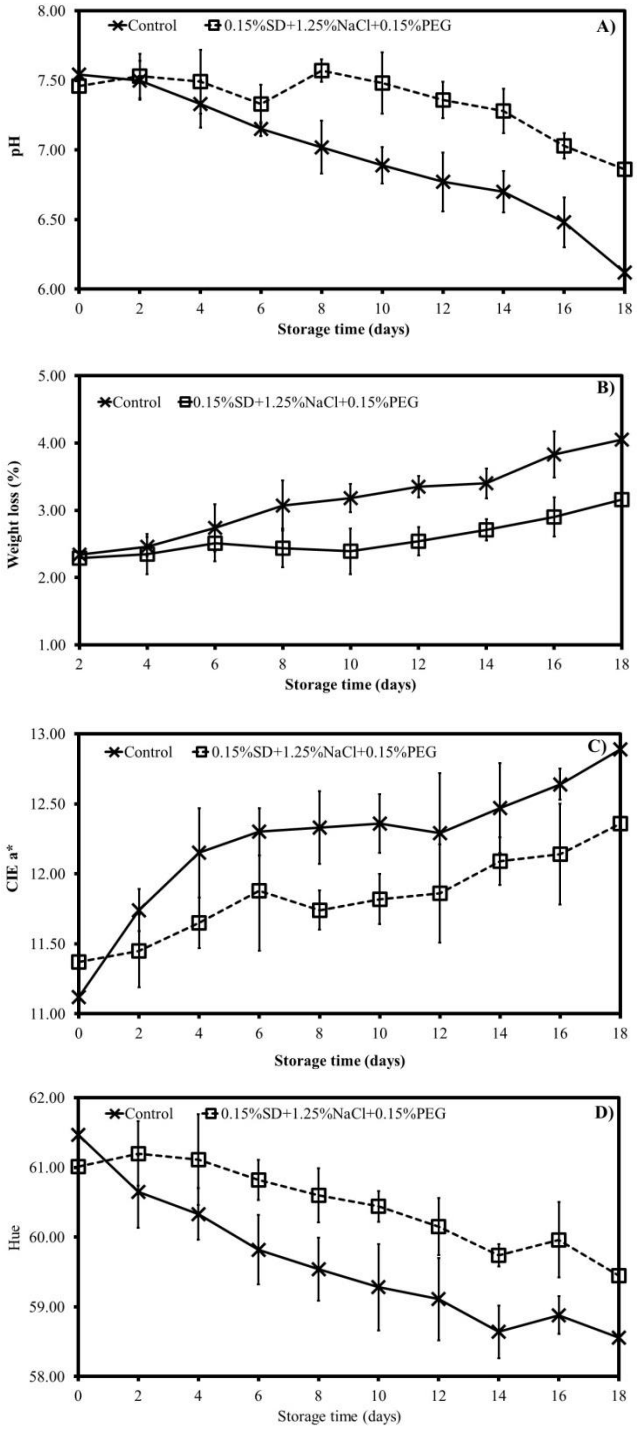

Figure 2. Effect of sodium diacetate (SD), sodium chloride (NaCl), and polyethylene glycol (PEG) combination on pH value **(A)**, weight loss **(B)**, CIE a*: redness **(C)**, and hue of cooked duck blood curd **(D)** stored at 7°C for 18 days in slaughter warehouse. a*: redness

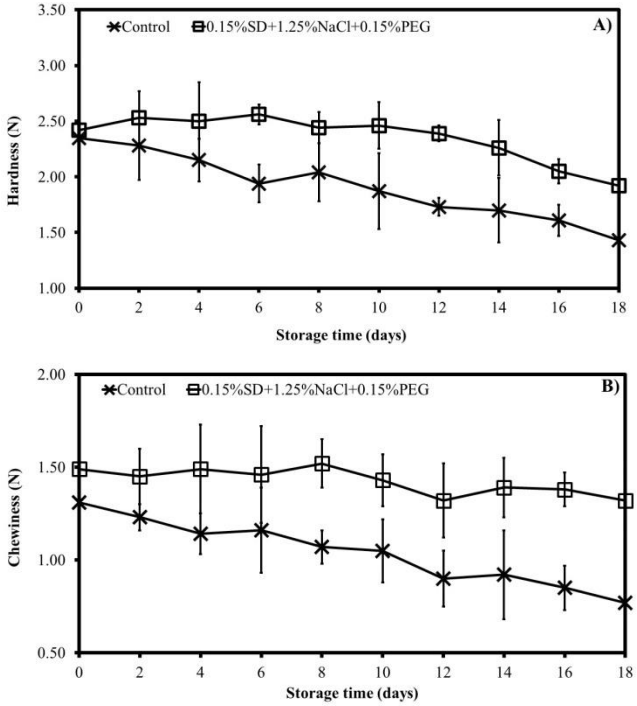

Figure 3. Effect of sodium diacetate (SD), sodium chloride (NaCl), and polyethylene glycol (PEG) combination on hardness **(A)** and chewiness **(B)** of cooked duck blood curd stored at 7°C for 18 days in slaughter warehouse.

CONCLUSION

This study indicated that the combined treatment of SD, NaCl, and PEG could significantly decrease spoilage and restrict the growth of pathogenic microorganisms in cooked duck blood curd. This antimicrobial action could decelerate the changes in physical quality and extend the shelf life of cooked duck blood curd. The current results showed the potential use of SD, NaCl, and PEG combination as an appropriate preservative in cooked duck blood curd, and its application in slaughter warehouses and distribution. For future research, the direct addition of SD, NaCl, and PEG combination in raw blood before the cooking process can be studied to reduce the concentration of combined antimicrobials.

DECLARATIONS

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Authors' contribution

Pussadee Tangwatcharin and Supaluk Sorapukdee created the idea and designed the study. Warraluk Teemeesuk collected data. Pussadee Tangwatcharin performed the statistical analysis and drafted and approved the final manuscript. All authors checked and confirmed the final analysis data and the final version of the manuscript before publication in the journal.

Competing interests

The authors have declared that there are no competing interests.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Consent to publish

All authors informed their consent before inclusion in the study.

Ethical consideration

All the authors checked for ethical issues such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

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Characterization and Typology of Traditional Poultry Farming Systems in Southern Niger

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ABSTRACT

An appropriate agricultural policy that integrates knowledge of endogenous poultry practices should enhance household resilience by contributing to food and nutrition security and sustainable development in developing countries. The current cross-sectional survey aimed to characterize poultry breeding systems and identify types of traditional poultry farmers in Maradi and Zinder in southern Niger. Therefore, 600 households were investigated for the socio-economic parameters of poultry farmers, the breeding methods, the zootechnical parameters of the local chicken, and the health parameters relating to biosecurity and animal care. The results of the descriptive analyses indicated that traditional poultry activity is mainly carried out by men (73.5%) and small farmers (74.2%). Breeding management was primarily free-range breeding (99.3%). The majority of the surveyed herders (67.8%) were illiterate. However, 41.5% of them attended traditional Islamic Koranic schools. Most farmers (80%) were small-scale livestock farmers with an average herd size of 22 ± 24.9. The poultry raised were 93.3% local breeds, with chicken domination (66%). The housing did not meet the required standards, and the feed was mainly cereals. The female chicken can potentially produce 12.64 fertile eggs per clutch and brood 3.53 times per year. The leading cause of mortality in poultry was avian diseases (93.7%) and Newcastle disease in some cases. Poultry vaccination against Newcastle disease was reported by 31.5% of respondents. Of the respondents, 20% have partially observed hygiene and biosecurity measures. About 35.5% of the participants reported the provision of veterinary care, while 44% used phytotherapy to prevent or treat poultry diseases. Based on the results of this cluster analysis, three classes of poultry farmers were distinguished, each with specific characteristics. Poultry farmers in class 1 were particularly characterized by the diversity of their main activity and their level of education, those in class 2 were mostly employed in agriculture and had little school experience, and those in class 3 were characterized by their low level of vaccination practice and their lack of therapeutic animal care. The results also indicated that 15.7%, 70.8%, and 13.5% of poultry farmers belonged to classes 1, 2, and 3, respectively.

Keywords: Characterization, Farmer, Niger, Poultry diseases, Poultry production

INTRODUCTION

Niger is a Sahelian country by excellence. It faces recurrent food crises, forcing the government to consider political and institutional solutions to provide definitive responses. In 2012, the government developed and implemented a strategy for sustainable food and nutritional security and agricultural development that includes the promotion of short-cycle livestock systems (poultry and fish farming) as one of the production's priority investment programs (HCI3N, 2012).

In Niger, poultry production is dominated by the traditional system. Of the population, 80% practice poultry farming, and 98% come from the traditional sector (MAG/EL, 2020). Similar to other developing countries, traditional poultry farming in Niger plays an important socio-cultural, nutritional and economic role. It is a means of improving food security (Wong et al., 2017) and nutrition (Scanes, 2007), alleviating poverty (Dolberg, 2003), creating employment and income, and contributing significantly to improving the living standard of poor populations (Fasina et al., 2007).

Despite its potential, traditional poultry farming faces many performance challenges, as well as biosecurity and husbandry barriers (Moula et al., 2012; Alem, 2014). The development of any strategy to promote the growth of the

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traditional poultry sector must be based on reliable and updated statistical data. These data constitute a decision-making means. However, data on the current situation are not widely available and can only be obtained through better monitoring of farms and better collection and analysis of field data. Over the past decade, few studies have documented traditional poultry farming in Niger. These studies focused on diseases in local chickens and guinea fowl (Idi et al., 1999; Idi et al., 2001; Souley et al., 2021) and characterization of local chickens (Moussa et al., 2020). However, none of these studies addressed the rearing system. The breeding system results from interactions between humans, their environment, and the flock. The study of breeding systems aims to account for the diversity of breeding practices and understand and analyze the animal's performance without blaming the delay on the producers or the inefficiency of knowledge transfer (Lhoste, 1984; Dedieu et al., 2008).

Thus, the present study aimed to advance the documentation of traditional poultry farming in Niger and to provide decision-makers with the information needed to guide research and development actions. Specifically, it described the characteristics of traditional poultry farming systems and highlighted the different types of poultry farmers, management methods, and the main factors and practices involved. Finally, the study provided relevant data to guide traditional poultry development projects and programs.

MATERIALS AND METHODS

Study area

The study was conducted in southern Niger, in 10 departments and 2 cities in the regions of Maradi and Zinder. These 12 administrative entities are covered the Sahelian and Sahelo-Sudanian agroecological zones.

The Sahelian zone is a steppe area with a Sahelian climate that covers the Departments of Mirriah, and Takeita, the Northan Departments of Aguié, Gazaoua, and Tessaoua, and the city of Zinder. The Sahelo-Sudanian zone is the savanna domain that covers Guidan Roumdji, Madarounfa, Kantché, Magaria, Dungass, the southern part of the departments of Aguié, Gazaoua, and Tessaoua, and the city of Maradi. This part of the territory is a transition zone between the Sahelian and Sudanian zones (Wata et al., 2012).

Zinder rain station records an average rainfall annual of 502.12 mm in 43 days, an average temperature ranging from 22.18 to 35.3°C, and an annual relative humidity ranging from 20.4 to 49.8% (INS, 2020). Maradi rainfall station records an average annual rainfall of 562.8 mm in 45 days, an average temperature ranging from 21.54 to 35.38°C, and an annual relative humidity of 27 to 57.6%. Rainfall occurs between June and September (INS, 2020).

The two study regions have an area of 197,574 km², or 15.6% of the national territory, with an estimated population of 9,584,421 inhabitants in 2021, representing 40.6% of the total population (INS, 2019).

Administratively, Niger is subdivided into regions, regions into departments and cities, departments into urban and rural communes, and cities into boroughs. Communes and boroughs are, in turn, subdivided into administrative villages, hamlets, camps, and neighborhoods. Figure 1 clearly illustrates the study area.

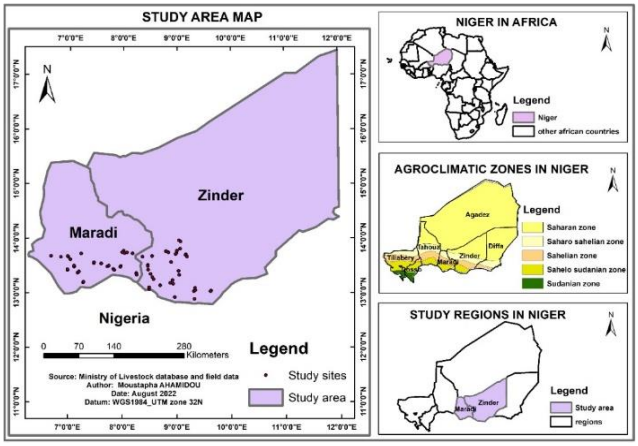

Figure 1. Study area of Southern Niger

Sampling

A stratified random sampling method with proportional allocation was used to define the samples. In the first stratum, the agricultural departments located along the border with the Federal Republic of Nigeria were selected in each of the two regions as well as the cities of Maradi and Zinder. In the second stratum, a maximum of three communes or boroughs were selected per department or city. In the next stage, a maximum of three localities were randomly selected in each commune or borough. Then, by referring to the national directory of localities (ReNaloc), the number of farm households to be surveyed per commune or borough was determined in proportion to the number of total farm households. Finally, the households surveyed were drawn from the list of households drawn up by the village chief. Table 1 shows the distribution of households surveyed.

Data collection

Data were collected from November 2021 to January 2022, including semi-open and closed-ended questions. The questionnaire was administered face-to-face using the

KoBoCollect collection tool (Version 2021.2.4). Information collected included geographic location, household socioeconomic data (gender, age, main activity, education level, poultry training, husbandry goals, and experience), farm technical data (species, breed, origin of animals, type and size of farming, habitat, feed, breeding parameters), and farm health data (mortalities and causes, knowledge of Newcastle Disease, biosecurity, vaccination practice, types of treatment, and access to husbandry service). Information on plants used by poultry farmers to prevent and treat poultry diseases was obtained in local languages, and the scientific names were checked in the lexicon of Niger plants written by Peyre de Fabregue (1979).

Table 1. Number of households surveyed by commune or borough in Niger from November 2021 to January 2022

Region	Departments and cities	Communes and boroughs	Number of households surveyed
Maradi	Aguié	Aguié	32
		Tchadoua	18
	Gazaoua	Gangara	11
		Gazaoua	23
	Guidan Roumdji	Chadakori	23
		Guidan Roumdji	19
		Tibiri	22
	Madarounfa	Dan Issa	19
		Djirataoua	16
		Serkin Yamma	9
	Tessaoua	Hawandawaki	9
		Maijirgui	14
		Tessaoua	31
	Maradi	Maradi 1	3
		Maradi 2	2
		Maradi 3	3
Zinder	Dungass	Dungass	34
		Dogo dogo	16
		Malawa	23
	Kantché	Matamey	12
		Kantché	12
		Yaouri	14
	Magaria	Bande	32
		Magaria	27
		Sassoumbroum	19
	Mirriah	Dogo	32
		Droum	29
		Mirriah	18
	Takeita	Dakoussa	16
		Garagoumssa	14
		Tirmini	30
	Zinder	Zinder 1	5
		Zinder 2	4
		Zinder 5	9
Total			600

Statistical analysis

To analyze the data, R software (Version 4.1.2) was used. The descriptive analysis was run to characterize the breeding systems by calculating the means, standard deviations, and relative and absolute frequencies. To carry out the typological study, Mixed data factor analysis (MDFA) was first used to combine the quantitative and qualitative variables into a single analysis and to identify subsets of homogeneous and strongly related variables according to the method of Pagès (2004). This method was performed using the ClustOfVar algorithm, a package of R. Secondly, principal component analysis (PCA) was applied to the synthetic obtained variables to identify those that can participate in the classification of the investigated poultry farmers. This approach resulted in an appropriate classification with a fairly acceptable explained variance. At this level, a step-by-step top-down method was applied. For the selection of synthetic variables, when the PCA is compiled, the variable that is poorly represented and/or contributes less to the construction of the principal components is eliminated. This means that this synthetic variable was nonsignificant ($p > 0.05$). The process was

repeated several times until an acceptable PCA was obtained. The synthetic variables retained in the PCA were subjected to Hierarchical ascending classification (HAC). Finally, the results of the variable classification were interpreted using the method by Kuentz-Simonet et al. (2013).

RESULTS

Socioeconomic characteristics of poultry farmers

The obtained results of the present study showed that traditional poultry farming is an activity dominated by men (73.5%) and small farmers (74.2%). The age of the poultry farmers surveyed ranged from 12 to 92 years, with an average of 39.45 ± 15.02 years. Most of them (67.8%) had no school experience. However, a large proportion of them (41.5%) had received Islamic education at the Koranic school. Regarding marital status, 86.2% of the herders surveyed were married. Regarding the years of experience in poultry farming, more than half (54.84%) had 1-10 years of experience (Table 2). About 53% of the households surveyed kept poultry for consumption plus income, 25% for income, 14% for ritual and tradition purposes, and 8% for consumption only.

Breeding methods and animal composition

In the case of the surveyed households, scavenging system (78.5%) and mid-scavenging system (20.8%) were the dominant breeding methods. In the scavenging system, the animals are not confined and can roam over long distances in the village. In contrast, in the mid-scavenging system, the animals are confined to their concessions and rarely have access to the village. Most of the poultry farmers surveyed (80%) could be classified as small-scale farmers with an average of 22.10 ± 24.9 head. The birds raised were mostly (93.3%) local breeds, and the basic stock was mainly acquired from local markets (64.8%), neighboring villages (29.8%), and traditional hatcheries (4%). The 87.2% of eggs laid by the hen were used for brooding. Approximately 60% of the farmers surveyed practiced single-species breeding, as opposed to 40% who combined chicken breeding with other species of domestic birds. The flock households were dominated by chickens (66%), followed by guinea fowls (22.2%), ducks (5.8%), pigeons (5.2%), and turkeys (0.5%).

Shelter and breeding equipment

In the traditional breeding system, the chicken house provides bird shelter at night and protects them from bad weather and predators. The findings of the present study indicated that nearly a quarter (22.7%) of the farmers surveyed did not have a chicken coop. In these households, the animals find shelter at night in the corners of houses in unfinished rooms on walls and trees. Among households with chicken coops (77.3%), three types of coops could be distinguished. The most common coops were made of straw huts (60%), improved coops were made of wood (30%), and wire coops (10%). In almost all cases, the breeding equipment was limited to the drinking trough, which served as a feeder. There are practically no feeders. However, the troughs are often made of pottery pieces, fragments of worn tires, or other troughs of circumstance.

Feed

Poultry feed was composed of cereals, including millet, sorghum, millet bran, wheat bran, kitchen waste, and water during the dry seasons (cold and hot). During the rainy season, it was made of birds' peck at insects and vegetation in addition to grain and water. The drinking water for poultry came mainly from wells, boreholes, and ponds.

Reproduction

Table 3 tabulates the mean for age at maturity of a cock, age at the first laying of a hen, number of eggs per laying, number of eggs hatched per laying, and number of layings per hen per year. The p-values of these reproductive parameters between the two agroecological zones were 0.82, 0.98, 0.36, 0.67, and 0.82, respectively. The difference in the means of the reproductive parameters between the two agroecological zones was nonsignificant ($p < 0.05$). Therefore, the agroecological zone factor did not affect the reproduction parameters.

Advisory and technical support for poultry farmers

Few of the farmers surveyed (9.7%) had received short-term training in poultry farming. The state designed a community animal health system and its partners to extend the network of veterinary services and bring the breeding service closer to the breeders. Thus, in 2003, the rural veterinary clinic and its network of auxiliaries were created in Niger. Basic animal health services were then provided by the technical breeding services, the private veterinary services of proximity, and especially by the breeding auxiliaries. Farmer schools' field are also set up to disseminate technological innovations and improve local practices in small-scale breeding. Poultry kits were also distributed to enhance poultry production or

distribute genetic materials. These operations were usually accompanied by deworming and mass vaccination. A new concept of poultry enterprise is implemented. It involves re-training the poultry farmer in the poultry business by building rural mini-farms in compliance with technical standards in terms of the structure of buildings, density of birds, monitoring of the farms, and training the farmers with the necessary support.

Table 2. Socioeconomic characteristics of poultry farmers in Niger from November 2021 to January 2022

Variables	Modalities	Number of poultry farmers	Frequency (%)
Gender	Male	441	73.5
	Female	159	26.5
Age	Youth (Under 36 years old)	278	46.33
	Adults (36 to 60 years old)	268	44.67
	Old (Over 60 years old)	54	9
Marital status	Single	76	12.7
	Married	517	86.2
	Widowed	7	1.2
Level of education	Non-literate	107	17.8
	Literacy	48	8
	Koranic school	249	41.5
	Primary	100	16.7
	Secondary	91	15.2
	Higher	5	0.8
Years of experience in poultry production	1 to 5 years	133	22.17
	6 to 10 years	196	32.67
	11 to 20 years	170	28.33
	21 to 30 years	84	14
	Over 30 years	17	2.83
Principal activity	Agriculture	445	74.2
	Trade	40	6.7
	Public service	32	5.3
	Other activities	83	13.8

Table 3. Zootechnical parameters of local chickens in southern Niger from November 2021 to January 2022

Agroecological zones / Production parameters	Sahelian	Sahelo-soudanian	Mean
Age at maturity of a cock (months)	4.75 ± 0.66	4.77 ± 0.67	4.76 ± 0.67
Age at which hens start laying (months)	6.5 ± 0.62	6.5 ± 0.61	6.5 ± 0.61
Number of eggs laid per clutch	12.51 ± 2.58	12.71 ± 2.52	12.64 ± 2.54
Number of chicks hatched per clutch	10.39 ± 2.05	10.46 ± 2.16	10.44 ± 2.12
Number of broods per year	3.54 ± 0.49	3.53 ± 0.49	3.53 ± 0.49

Causes of mortality and knowledge of Newcastle disease

The poultry farms in the households surveyed were characterized by high mortality of chickens and guinea fowl of all categories caused by disease (93.7%), trampling (3.2%), predators (1.6%), and climatic hazards (1.5%). Almost all surveyed farmers (96.7%) were aware of Newcastle disease and could describe its clinical signs, such as greenish diarrhea, torticollis, respiratory distress, and depression, most often associated with high mortality of approximately 90%. Approximately 82.7% of the surveyed farmers stated that Newcastle disease occurred in the cold dry season. However, 12.7% of the surveyed farmers reported that it happened in the hot dry season, and 4.6% of the surveyed farmers stated that it appeared all year round. According to poultry farmers surveyed,

outbreaks of Newcastle disease occur mainly at the beginning and end of the cold, dry season, and the Harmattan wind spreads the disease.

Biosecurity

Biosecurity is the set of practices and measures used to prevent the introduction, maintenance, and dissemination of pathogens on a farm. The results of the current study indicated that only one-fifth of the farmers practiced quarantine of newly acquired animals (20.2%) and isolation of sick animals (19.2%). Approximately 70.8% of poultry farmers, discarded dead birds in the wild. Almost all farmers (99%) swept the poultry house, but with a wide range of frequency. Only 1% of the poultry farmers swept the barn daily, 60% swept weekly, 16% swept monthly, and 23% rarely. The majority of poultry farmers (60%) washed water troughs weekly. However, only 22% used soap or detergent for washing. The sanitary vacuum is a sanitation operation on the farm that includes disinsectization, deratting, cleaning, and disinfection of the poultry house and its surroundings. In the present study, 17% of the farmers practiced sanitation by changing the location of the barn, spreading hot ash in the barn, or incinerating the barn. Approximately 31.5% of the farmers stated that they had vaccinated their animals at least once and only against Newcastle disease. Vaccination of poultry against Newcastle disease usually occurs during the free vaccination campaign for livestock (cattle, sheep, goats, and camels) in December. This prophylaxis operation is carried out by state services and private actors, including breeding auxiliaries, for 0.09-0.17 USD per poultry. Nevertheless, periodic vaccination operations are organized and financed with the support of non-governmental organizations and livestock development projects in their areas of intervention.

Animal care

To treat poultry diseases, 85% of the poultry farmers responded that they provided therapeutic care to the animals. Of these poultry farmers, 35.5% provided veterinary care, 44% used ethnoveterinary methods, 15.5% combined both methods, and 5% used non-conventional treatment with human products. Fake veterinary products were highly accessible to farmers. Due to the porous borders, they were sold in weekly markets at a very affordable cost and came from neighboring countries. The most popular medications were Antiparasitics, dewormers, antibiotics, and vitamins in tablets or sachets. The human medicines were mainly Paracetamol, Metronidazole, and Amoxicillin. Traditional knowledge of medicinal plants was commonly used for animal treatment. The herders used different parts of the plant (leaves, bark, root, stem, seeds, and fruits). Some ingredients, such as chili and ash, were also used in the treatment and deworming of poultry. A total of 17 plants from 12 commonly used botanical families were identified in this regard (Table 4).

Table 4. Plants commonly used by poultry farmers for the prevention and treatment of poultry diseases in southern Niger

Vernacular names	Scientific names and botanical families	Plant organs	Diseases
Dogo'n yaro	*Azadirachta Indica* (*Méliaceae*)	Leaves, bark, and seeds	Diarrhea, Mites, Infection
Dânia	*Sclerocarya Birrea (Anacardiaceae)*	Bark, leaves, roots	Diarrhea
Faru	*Lannea Fruticosa (Anacardiaceae)*	Bark, leaves	Diarrhea
Kukuki	*Sterculia Setigera (Sterculiaceae)*	Bark, root	Infection
Golo'n zaki	*Cucumis Metuliferus (Cucurbitaceae)*	Fruits	Apathy, Depression
Aguwa	*Euphorbia Balsamifera (Euphorbiaceae)*	Branch	Diarrhea, Newcastle disease
Ida'n sânyia	*Solamum Incanum (Solanaceae)*	Fruit, Leaves, Stem, Root	Newcastle disease, Mites
Ida'n zakara	*Withania Somnifera (Solanaceae)*	Leaves, root	Nervous disorders
Pfataka	*Pergularia Tomentosa (Apocynaceae)*	Leaves, root	Respiratory disorders
Duman kada	*Ipomoea Asarifolia (Convolvulaceae)*	Leaves	Diarrhea, Nervous disorders
Kafurdo	*Citrullus Colocynthis (Cucurbitaceae)*	Fruits	Diarrhea, Nervous disorders
Kiryia	*Prosopis Africana (Mimosaceae)*	Leaves, bark, root	Diarrhea, Anorexia, Depression
Bagaruwa	*Acacia Nilotica (Mimosaceae)*	Fruit, leaves, bark, root	Diarrhea, Mites
Raydoré	*Cassia Occidentalis (Caesalpiniaceae)*	Leaves, stem, flowers, roots	Prostration, Roundworm, Tapeworm
Gamjy	*Ficus Platyphylla (Moraceae)*	Bark, root	Apathy, Plague, Infection
Thiédya	*Ficus Sycomorus (Moraceae)*	Leaves, bark	Diarrhea, Respiratory disorders
Tum-iya	*Aerva Javanica (Amaranthaceae)*	Sheets	Diarrhea, Roundworm, Tapeworm

Access of poultry farmers to the breeding service

The results of the survey indicated that 97.7% of poultry farmers had physical access to the livestock service. Among these farmers, 53.5% had access to the public service, 18.5% used the private service of proximity, and 25.7% had access to both types of service.

Classification of variables

Following the factorial analysis of the mixed data and their compilation with the ClustOfVar algorithm, eight synthetic variables (SV) were identified based on the 14 source variables introduced, with a cohesion gain of 72.59%. The SV 1 represents the origin of the animals in relation to the type and size of breeding, SV2 corresponds to the agroecological zone, SV3 represents gender, SV4 reflects the main activity of the poultry farmer in relation to his level of education, SV5 corresponds to the poultry breed, SV6 reflects animal care in terms of variety of health care, disease management, and poultry house ownership. Synthetic variable 7 includes prophylaxis, management of dead poultry bodies, and vaccination practices, and SV8 corresponds to access to breeding services. After applying a step-by-step top-down selection based on the contribution to the axes and the quality of the representation of each synthetic variable in PCA, the SV3, SV4, SV6, and SV7 gave the best result with an explained variance of 59.16% of the total variance. Thus, these synthetic variables were selected to participate in the classification of breeding systems.

Classification of breeding systems

The application of the Hierarchical ascending classification (HAC) method on the synthetic variables retained in the PCA led to the classification of the farms into three classes with an explained inertia rate of 65.1%. Figure 2 shows the projection of the colored individuals according to their class in the factorial plane and indicates that the classes are homogeneous and separated from each other. Table 5 shows that the participation of each synthetic variable in the classification of the cluster is significant ($p < 0.05$). The measure of the intensity of the relationship between the cluster of poultry farmers and each synthetic variable is given by the value of Eta2. Eta2 is a Spearman correlation coefficient that measures the strength of the relationship between the synthetic variables and the clusters. When the value of Eta2 < 0.5, the relationship is weak. When the Eta2 value is between 0.6 and 0.7, the relationship is strong, and when the Eta2 > 0.7, the relationship is very strong. The Eta2 values of the synthetic variables SV4 and SV6 being higher than 0.7; these synthetic variables have strongly contributed to the classification of the poultry farmers groups.

Table 5. Relationship between the cluster variable of poultry farmers and associated synthetic variables

Variables	Eta^2	P-value
VS6	0.86302574	$p < 0.05$
VS4	0.75380497	$p < 0.05$
VS7	0.07860523	$p < 0.05$
VS3	0.03042297	$p < 0.05$

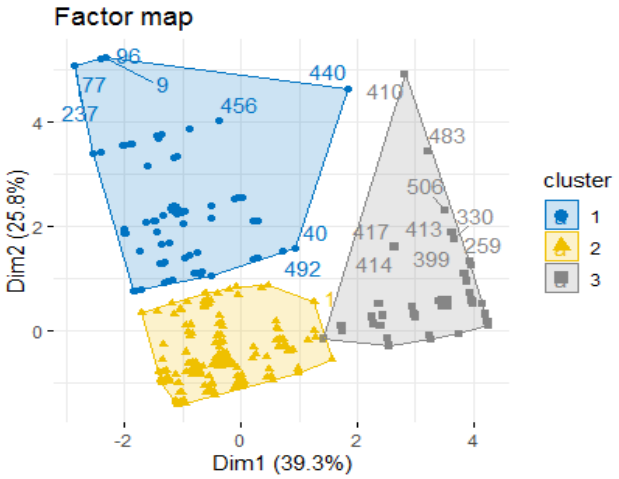

Figure 2. Graphical representation of the classes on axes 1 and 2

Class 1 represented 15.7% of the survey population and is dominated by men (85.11%). The poultry farmers in this class were mostly employed (73.41%) in the secondary and tertiary sectors. They belonged to four socio-professional sub-groups. The largest subgroup (36.17%) included several heterogeneous socio-professional categories, consisting of restaurant workers, pupils, motorcycle cab drivers, agricultural product processors, butchers, tailors, mechanics, carpenters, masons, blacksmiths, tire repairers, workers, marabouts, town criers and traditional practitioners. The second subgroup (29.79%) was made up of public service employees assigned by the state or recruited by the local authorities to serve in the surveyed localities. This subgroup comprised teachers, health workers, rural development workers (agriculture, breeding, and environment), and municipal workers. The third subgroup was farmers (26.6%), and the last and least important subgroup was traders (7.45%). In this class, all the herders were educated (100%). Most of these farmers (84.04%) had a shelter or a henhouse to house the birds at night. In

addition, more than half of them practiced vaccination (56.32%). Regarding the disease, 53.19% of them administered therapeutic care to the animals, and 42.55% opted for sanitary slaughter to minimize the risk of loss. However, some of them engaged in risky behavior by disposing of sick birds by sale (2.13%) or doing nothing (2.13%). Of those who treated sick animals, half (51.06%) used veterinary care, 13.83% used ethnoveterinary medicine, 24.47% combined both methods, and 10.64% used human medications. Regarding the management of dead poultry bodies, 72.34% of the farmers discarded the dead bodies in the wild, while 27.66% incinerated or buried the dead poultry bodies.

Class 2 had the largest number of poultry farmers studied (70.8%), with a dominance of men (73.41%). In this class, the majority of poultry farmers (84.47%) worked in the primary sector (agriculture) and were largely (80.71%) not in school. Most of these farmers (84.04%) had a shelter or a henhouse to house the birds at night. In addition, two-thirds of these farmers (66.65%) practiced vaccination. In case of disease, 61.41% administered care to the animals, while 37.18% eliminated the animals by slaughter. However, a minority of farmers tended to sell sick animals (0.71%) or observed sick animals without doing anything (0.71%). Regarding the types of therapeutic care, 38.82% of the poultry farmers administered veterinary care, 28.47% used ethnoveterinary medicine, 24.71% combined both methods, and 8% used human products. In this class, 64.24% of the farmers disposed of the dead birds in the wild, 34.35% buried or incinerated the dead birds, and 1.42% consumed the dead bodies.

Class 3 represented 13.5% of the total surveyed population. It was the class with the least number of farmers. In this class, there was a high proportion of women (40.74%). The majority of farmers (82.72%) were employed in the primary sector (agriculture). Considering literacy, 77.55% of them did not attend school. About 70.37% of the poultry farmers had a chicken coop. However, almost all the breeders (98.77%) did not vaccinate their animals. In case of disease, 7.41% of the farmers disposed of the animals by stamping out, 2.47% got rid of birds by selling, and 90.12% did nothing. Regarding the management of poultry corpses, 96.3% of the farmers left poultry corpses in the wild, and 3.7% incinerated or buried poultry corpses. The characteristics of the different classes of traditional poultry farmers in southern Niger are shown in Table 6.

Table 6. Characteristics of the different classes of traditional poultry farmers in southern Niger

Variables	Modalities	Class 1 (%)	Class 2 (%)	Class 3 (%)
Sex of the breeder	Male	85.11	73.41	59.26
	Female	14.89	26.59	40.74
Principal activity	Agriculture	26.6	84.47	82.72
	Trade	7.45	6.82	4.94
	Public service	29.79	0.71	1.23
	Other activities	36.17	8	11.11
Level of education	Koranic school	0	52	34.57
	Literacy	0	9.41	9.88
	Non-literate	0	19.3	32.1
	Primary	2.13	19.29	19.75
	Secondary	92.55	0	3.7
	Higher	5.32	0	0
Possession of a henhouse	Yes	84.04	84.71	70.37
	No	15.96	15.29	29.63
Vaccination practice	Yes	56.38	66.65	1.23
	No	43.62	34.35	98.77
Disease management	Treatment	53.19	61.41	0
	Sanitary slaughter	42.55	37.18	7.41
	Sale	2.13	0.71	2.47
	Nothing	2.13	0.71	90.12
Types of care	Veterinary medicine	51.06	38.82	0
	Ethno-veterinary medicine	13.83	28.47	0
	Combination of both methods	24.47	24.71	0
	Non-conventional treatment	10.64	8	0
	None	0	0	100
Management of corpses	Disposal in nature	72.34	64.24	96.3
	Incineration or burial	27.66	34.35	3.7
	Consumption	0	1.41	0

Class 1: 94 poultry farmers, Class 2: 425 poultry farmers, Class 3: 81 poultry farmers

DISCUSSION

The results of the current study revealed that the practice of traditional poultry farming is dominated by men (73.5%). This trend was previously reported in the results of the General Census of Agriculture and Livestock (RGAC, 2008), indicating that poultry breeding in Niger is practiced by 48% of men, 28% of children, and 24% of women. The findings of the current study are in agreement with the study conducted in Burkina Faso, where Pindé et al. (2020) reported that 70.26% of men practiced poultry farming. Conversely, a Zimbabwean study revealed that 88.9% of women dominated poultry activities (Ndiweni et al., 2013). Small-scale poultry breeding by women has been highly advantageous because the income generated through the sale of poultry and eggs would be under their control, which would enhance their empowerment and help households to overcome financial difficulties (Begum et al., 2019). Guèye (2005) indicated that poultry ownership results from the communities' socio-cultural and religious conditions in most rural areas of Africa.

The present results indicated that 74.2% of the households were engaged in agriculture as their primary activity. Similarly, Talaki et al. (2020) reported that 91.35% of poultry farmers in Togo are employed in the agricultural sector. In the study area, households involved in agriculture or other occupational sectors have diversified into poultry farming to supplement their income. Kalifa et al. (2018) noted that integrating poultry practice into farms is profitable. The reason is that poultry contributes to farm income and organic manure production to fertilize the fields. Poultry in rural areas is a means of saving income that can be easily mobilized to meet the basic needs of households. It serves as a social safety net and solidarity for rural populations (Melesse, 2014; Nahimana et al., 2019).

Regarding the herders' education level, it was found that 67.2% of the herders did not attend school. However, a high proportion (41%) attended Islamic teachings at the Koranic school. The current results contrast with the study conducted in Côte d'Ivoire, where Etienne et al. (2021) recorded that 52.5% of herders had no experience of attending school. The Koranic school is one of the most popular types of school in the Muslim-majority countries of Sub-Saharan Africa. It constitutes the only training and literacy offered for populations marginalized by the formal education system (Meunier, 1995; Stefania, 2003).

The results of the present study contradict the one conducted by Ebwa et al. (2019) in the Democratic Republic of Congo, indicating that 77% of poultry farmers raised poultry solely for consumption.

Regarding the breeds of poultry raised and the predominance of chicken, the present results are consistent with the study conducted in Burkina Faso by Bansé et al. (2017), reporting that almost all producers raised poultry of local breeds with 66% chickens.

Age at the first laying of a hen, number of eggs laid per clutch, number of eggs hatched per brood, and number of egg laying per hen per year are almost identical in the two agroecological zones. With respect to the age at first laying, the present results are close to those reported by Dzogbema et al. (2021) in Togo and Ayssiwede et al. (2013) in Senegal, recording 6.76 and 6.38, respectively. Regarding the number of eggs laid per hen per clutch, the findings of the current study were close to those of Mauritania and Ethiopia, where Ahmed and N'Daw, (2015) and Dassie and Ogle (2001) reported 12.96 and 12.9 eggs per hen per clutch, respectively. The number of eggs hatched per clutch by Dassie and Ogle (2001) in Ethiopia was similar to that of the present research. Concerning the number of broods per hen per year, the obtained results of the current study were comparable to those reported by other authors in Africa (Mwalusanya et al., 2002; Fotsa, 2008; Ayssiwede et al., 2013; Ahmed and N'Daw, 2015; Kibreab et al., 2016; Dzogbema et al., 2021). Generally, the number of broods per hen per year for indigenous African chicken ranges from 3 to 4 (Ahmed and N'Daw, 2015; Kibreab et al., 2016; Dzogbema et al., 2021). However, Mammo et al. (2008) reported a clutch number per hen of 5.06 ± 1.65 per year in Ethiopia.

Avian diseases were the main cause of mortality (93.7%) in the present study. This finding is in agreement with several previous studies. Meskerem (2019) and Otte et al. (2021) reported 67% and 60% disease-related poultry loss in village poultry farming in their studies in Ethiopia and Tanzania, respectively. Meskerem (2019) and Otte et al. (2021) also reported that Newcastle disease accounted for 64% and 54% of the identified diseases.

Regarding animals' origin, the present results are in agreement with a previous study conducted in Nigeria, where Ameji et al. (2012) reported that 64.7% of poultry farmers obtained their breeding stock from the live bird market and other unknown sources.

Present results are close to the study results conducted in Nigeria, where Ajewole et al. (2014) reported that 27.5% of the farmers practiced the quarantine of new birds. As for the isolation of sick birds and regular cleaning of the birds' environment, the current study

contrasts with Ajewole et al. (2014), where 100% of the surveyed farmers regularly cleaned the birds' habitat and practiced the isolation of sick birds. Ndem and Ogba (2017) indicated that the isolation of sick birds and corpses, movement control, and sanitary practices could be used effectively to prevent poultry diseases.

In the present study, 70% of the poultry farmers surveyed were disposing of dead poultry bodies in the wild. Similar observations were made in a previous study conducted by Abdurrahman et al. (2016) in Nigeria. These authors revealed that 50% of farmers disposed of dead poultry bodies by dumping them in landfills. Guittet et al. (2018) indicated that this practice is not without risk of the contamination of susceptible animals, the case where the birds' dead bodies are infected.

In terms of medical prophylaxis, 31.5% of the poultry farmers surveyed stated that they had vaccinated their animals only against Newcastle disease. This percentage is relatively low. The emphasis on vaccination against Newcastle disease is justified. This is the most important disease in traditional poultry production. However, the current results agree with those of a previous study conducted in Kenya, where Ezra et al. (2020) reported that 35% of poultry farmers reported having vaccinated their animals. Bessell et al. (2017) indicated that implementing a strategy based on the distribution of vaccines and community awareness of good breeding practices helps to increase the vaccination rate. The traditional system of treating animal diseases is still widely used in remote rural areas where modern veterinary care facilities are scarce or deficient (Phondani et al., 2010). The low cost of ethnoveterinary medicine makes it an alternative to modern medication for traditional poultry farmers (Adebayo et al., 2020). Several authors have documented the use of medicinal plants in the management of poultry diseases (Lagu and Kayanja, 2010; Meskerem, 2019). Some of the plant species identified in the present study have been reported in previous studies with scientific evidence of their use in poultry. The extracts of these plants, including *Acacia nilotica*, *Withania somnifera*, and *Azadiractha indica* have biological activities against *Escherichia coli*, *Staphyllococcus aureus*, *Klebsiella pneumoniae* and against Newcastle disease and fowl pox viruses (Mohamed et al., 2010; Ashraf et al., 2018). They also act on external parasites and helminths (Carine et al., 2021).

Although the government's policy of bringing the breeding service closer to the breeders, and the surveyed farmers had the best physical access to the breeding service (97.7%), the use of veterinary care (35.5%) and vaccination (31.5%) were relatively low. A previous study conducted by Asfaw et al. (2021) in Ethiopia found that poultry health services are at minimum due in part to the lack of organized private poultry health service providers available to all and the low financial means of rural poultry farmers to hire private veterinarians. In many rural areas of low- and middle-income countries, the size of the area to be covered and the lack of resources and infrastructure can limit veterinary and extension services. Veterinary services are mostly devoted to crop or ruminant production, with little health care or advice accessible to small-scale poultry farmers (Wong et al., 2017). According to Enahoro et al. (2021), it is essential to improve the distribution channels of good quality drugs and vaccines, strengthen the technical level of poultry farmers, and support stakeholder involvement mechanisms to improve community-based poultry care services.

The typological analysis allowed us to meet one of the objectives of this study, which is to classify traditional poultry breeding farms according to the values of the synthetic variables taken into account in the classification and the significant links established between the groups of individuals. The results of the present study differ from those obtained by Dzogbema et al. (2021), and Pindé et al. (2020) in Togo, and Burkina Faso with regard to the objectives of the study and the variables introduced for the analysis.

CONCLUSION

This survey explored the characteristics of traditional poultry breeding systems in southern Niger and highlighted the variety of breeding systems by considering the socioeconomic aspects of breeders and animal health management (biosecurity, animal care). The main constraints to the current state of production of these types of breeding systems have been identified. To improve the current condition, future research is needed to focus on the control of poultry diseases, particularly Newcastle disease, to reduce mortality. Moreover, more studies should be conducted on adaptable and sustainable biosecurity measures for family poultry farming to reduce the risk of contamination; improvement of the habitat, poultry feed and breeding methods to optimize production; development of veterinary ethnomedicine as an alternative to modern medicine; capacity building for all stakeholders; and dissemination of good poultry practices.

DECLARATION

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Authors' contribution

This work was carried out by the contribution of all authors. Ahamidou Moustapha designed the protocol, collected and analyzed data, and draft the manuscript. Akourki Adamou validated the protocol, supervised the data collection and revised the manuscript. Essodina Talaki validated the protocol, supervised the data collection and revised the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

The authors of the current study have checked ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy).

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The Importance of Poultry Meat in Medicine: A Review

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ABSTRACT

The animal products, such as meat, milk, skin, blood, honey, and urine, have medicinal value for human diseases. Due to having high-quality components, poultry meat has therapeutic value. The present review aimed to describe the medicinal values of poultry meat for individuals who consume it during their life. Most poultry meat is classified as white meat, which contains lower fat and higher protein, compared with the meat of ovine, bovine, and pig. This feature of poultry meat (lower fat and higher protein) helps its consumers to have a normal physiological function of different organ systems. Moreover, it prevents many non-infectious diseases, including overweight, diabetes, and cardiovascular diseases. Selenium and low contents of carcinogenic substances (myoglobin, heme iron, and saturated fat) in poultry meat also prevent different types of cancers. Poultry meat is also recommended to avoid anemia, cardiovascular diseases, and diabetes. Dietary proteins, vitamins, and minerals in chicken meat are used for anti-aging, developing muscle and bone, improving the immune system, and increasing brain function. Traditionally, poultry is recommended as a supportive treatment for respiratory diseases, such as the common cold. Thus, consumption of poultry meat, especially chickens, up to 300g/once a week is recommended to prevent and reduce the risks of gastrointestinal cancers such as oesophageal cancer. Generally, regular consumption of poultry meat has health benefits for humans to prevent and reduce the risk of different diseases as chicken meat is a rich source of nutrition that can enhance the immunity system and tackle human disease risk factors.

Keywords: Consumption, Health benefits, Meat, Poultry

INTRODUCTION

Human being reared different types of bird species, including chicken, ostrich, guinea fowl, duck, and turkey, to gain meat sources (Abafogi et al., 2022). Most poultry meats are categorized as white meat, while the meat of other domesticated animals is classified as red meat. Chicken meat contains the most valuable proteins and amino acids for human health. Several scientific studies conducted in different parts of the world on different food consumption habits with varying taboos of food indicated the relationship between human nutrition and human health (EFSA, 2017; Ambaw et al., 2021). Many investigations support the correlation between the consumption of chicken meat and the prevention of cardiovascular diseases and their risk factors, such as obesity, diabetes mellitus, and cancers (EUP, 2019; Aditya, 2020). The meats of poultry are essential sources of beneficent diet for human health. The high contents of protein, vitamins, minerals, and low contents of lipid have made chicken meats beneficial for people of all ages (Franca et al., 2015). Chicken meat contains all necessary amino acids, including cartilage proteins and tissue-building materials. The large amount of minerals in chicken meat supports the blood, cardiovascular, and nervous systems (EUP, 2019). The low cholesterol and fat content make chicken meat real salvation for those suffering from problems with blood vessels (Gordana et al., 2018). This review aimed to explain the beneficial effects of poultry meat on human health.

Obesity

Recent studies have observed the importance of chicken meat consumption for controlling and preventing obesity (Astrup et al., 2014). Generally, consuming dietary protein obtained from poultry meat effectively reduces

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obesity because poultry meat has high protein and low-fat content (Marangoni et al., 2015; Metin and Orkide, 2017). The report mentioned that the risk of obesity was lower in individuals who consumed food containing low carbohydrates and was rich in protein compared with other types of food with a high amount of carbohydrates and low amount of protein content (Stoica et al., 2020). The reason is that protein with high satiety leads to minimizing the consumption of sugar, glucose, and different sweet foods, so humans eat a low amount of calories per day (Astrup et al., 2014).

Poultry meat consumption within 5-6 months results in weight loss due to low contents of carbohydrates and high amounts of proteins (Paoli, 2014). This mechanism could result in increased satiety, followed by fewer calorie consumptions during subsequent meals and decreased carbohydrate consumption within dietary regimens containing higher proportions of proteins (Astrup et al., 2014). It was also claimed that these mechanisms could be a synergistic effect. In addition to their satiety-producing effect and prevention of carbohydrate consumption, proteins are also responsible for higher thermogenesis through increasing protein synthesis and adenosine triphosphate utilization related to peptide bond formation as well as urea formation and glucose synthesis from other types of nutrients (Westerterp et al., 2009). The intake of protein in substitution of the same amount of carbohydrates decreases the overall glycemic diet, which results in the control of overweight (Promintzer and Krebs, 2006).

Cardiovascular diseases

Protein consumption greatly impacts well-being and normality of the cardiovascular system (Hu, 2005). Poultry meat is a proper diet for reducing the risk of developing diseases related to the blood circulation system, including heart and blood vessel diseases. The collagen produced from poultry is used to control hypertension (High blood pressure, Marangoni et al., 2015; López et al., 2019). Saturated fat and cholesterol are the main risk factors causing atherosclerosis, cardiovascular diseases, hypertension, and increased blood cholesterol (Abete et al., 2014). Due to low contents of saturated fat and cholesterol, the risk of occurrence of those diseases would be decreased by 19% when poultry meat is replaced as a meal with other meat (Bernstein et al., 2010). Previous studies in America on women indicated a negative association between the consumption of chicken meat and the risk of cardiovascular diseases (Hu et al., 1999). The research carried out more than two decades ago indicated a positive correlation between the frequent consumption of chicken meat and the health condition of individuals (Feskens et al., 2013). The reason could be the minimized Na^+(Sodium), Fe (heme iron), and more polyunsaturated fats in meals. Therefore, poultry meat is a great solution and an effective strategy for reducing cardiovascular disease (Hu, 2005). Due to the high content of Niacin, poultry meat helps the body generates energy and produces red blood cells (Adebowale, 2019). Niacin is taken as therapy in individuals with a history of hyperlipidemia (Keene et al., 2014; Garg et al., 2017). Niacin is an effective medication for cardiovascular diseases, reducing the risk of sudden death due to heart and blood vessel diseases (Duggal et al., 2010). Thus, poultry meat consumption is an important cause in reducing the risks of heart attack, hypertension, and other cardiovascular diseases (Adebowale, 2019).

Diabetes

Recently, it has been found that food consumption style is the foremost important factor for developing or preventing metabolic diseases, such as diabetes (Sami et al., 2017, Martín-Peláez et al., 2020; Guo et al., 2020). Subsequent food consumption increases or decreases the risk factors of diabetes in humans. The consumption of saturated fat originating from animal fat is among the most crucial risk factors for type 2 diabetes (Feskens et al., 2013). The positive association between the consumption of lipids and insulin resistance and, therefore, frequent consumption of red meat are the main risk factors for type 2 diabetes (Pan et al., 2011).

Even though the frequent consumption of animal-origin protein represents a risk of diabetes, the intake of poultry as a way of balanced food is advisable for reducing the development of metabolic disease in society (Esposito et al., 2010). A healthy lifestyle, which includes consuming poultry meat and plant-originated food, is related to minimizing the risk of death in individuals with diabetes (Sluik et al., 2014). These findings support the adjustment of lifestyle and food intake habits, within which poultry with low content of saturated fat provides a healthier alternative to animal protein ingestion in daily food, so it is suggested as an indication of a healthy diet (Enkhmaa, 2018).

Previous studies have revealed the effect of lifestyle interventions on the decreased possibility of type 2 diabetes by reducing numerous risk factors, including too much ingestion of fat, especially saturated fat (Pan et al., 2011, Lee et al., 2013; Rice Bradley, 2018). It was indicated that for individuals who consumed a high

amount of animal proteins, the incidence of diabetes was higher (Van Nielen et al., 2014). However, studies on the ingestion of chicken meat have proved the insignificant association between the frequent ingestion of poultry meat and diabetes (Feskens et al., 2013). Frequent and enormous ingestion of poultry meat could effectively prevent diabetes type2 (Esposito et al., 2010). The finding from the European prospective investigation into cancer and nutrition (EPIC) research indicated that following a healthier lifestyle and ingesting chicken meat with other plant-originated food is associated with a reduction in the death rate in diabetic patients (Bingham and Riboli, 2004). Thus, it is confirmed that diabetic patients can achieve significant profits from an overall lifestyle modification, including chicken meat ingestion (Sluik et al., 2014).

Cancer

Different types of cancer which occur in the gastrointestinal organ are highly related to the ingestion of red meat (Gordana et al., 2018). Myoglobin found in red meat could generate precancerous tumors through the catalytic impact of heme iron on the creation of carcinogenic N-nitroso compounds and the formation of cytotoxic and genotoxic aldehydes through lipid peroxidation (Turesky 2007 and Bastide et al., 2011). These potentially harmful contents of red meat (myoglobin, saturated fat, heme iron, sodium, N-nitroso compounds, and aromatic amines) formed by high-temperature cooking, as a result of red meat is the main factor for the occurrence of cancers (Gordana et al., 2018). Thus, it confirmed that excessive intake of red meat is a significant risk factor for the incidence of cancer in different parts of the body. The amount of harmful substance in red meat which causes cancer is higher than in white meat. Thus, poultry meat has a low amount of myoglobin, compared to other types of meat. Thus, frequent intake of red meat increases the chance of occurrence of cancers, while consumption of poultry meat indicates a negative association with the development of cancer in different parts of the human body, which includes hepatocellular carcinoma, ovarian tumor, lung cancer, oesophageal cancer (Salehi et al., 2013). There is a negative association between the frequent ingestion of chicken meat and the risk of developing oesophageal cancer. In addition, the occurrence of esophageal cancer is reduced by about 53% in Europe through frequent consumption of 300 g/week of poultry meat (Zhu et al., 2014). The study carried out by the Mario Negri Institute of Milan in the late 1990s in three regions of Northern Italy (Milan, Padua, and Pordenone) showed that poultry meat ingestion was associated with reducing the risk of occurrences of oesophageal cancers (Bosetti et al., 2000).

Another research revealed that the development of breast cancer in women was inversely associated with their history of chicken meat consumption (Bingham and Riboli, 2004). The investigation about the effects of different food sources on the development of cancer indicated that replacing an equal amount of daily consumption of red meat with poultry meat could reduce the risk of breast cancer by 17-24% in women and reduce the risk of lung cancer by 10% (Farvid et al., 2014).

Body function

Poultry meat is enriched by different essential mineral, which includes Ca^{+}(calcium), Mg^{+}(magnesium), P^{-} (phosphorus), and Na^{+} (sodium) when compared with other red meat (Marangoni et al., 2015). An almost similar amount of iron (0.97-1.04 mg/100g) is found in pork and poultry meat. Iron is needed to form hemoglobin, used as remedies for anemia and regular muscle activity. Calcium and phosphorus are essential for normal bone activity and formation. The phosphorus in chicken meat needs to maintain the normal skeleton system, central nervous system function, teeth care, and metabolic function. Sodium is an electrolyte, and magnesium is input for the normal formation of protein and muscle functions. While selenium is found in high amounts in chickens (8.6 µg to 41 µg/100g), frequent consumption of chicken meat (55µg per day) could increase metabolism rates, particularly the thyroid hormones, antioxidant defense system, and immune system of the body (Surai et al., 2018).

Of all types of vitamins in poultry meat, vitamin B3 (Niacin) is found in the highest amount, and the amount of vitamins A and B6 is also higher in poultry meat than in other animals. Niacin is essential for the normal metabolism of carbohydrates and energy generation. Its function is to prevent problems such as cataracts, various skin diseases, and the nervous system. Niacin is responsible for the synthesis of nutrients of sex hormones and gets a better circulation system, and reduces blood cholesterol (Garg et al., 2017). The persistent shortage of Niacin in humans and animals causes pelagic disease, which is identified by abnormal skin pigmentation (skin redness), gastrointestinal disorder, and abnormality of brain function or dementia (Hegyi et al., 2004). Therefore, chicken meat can be used to conveniently access primary sources of nutrients, vitamins, and minerals necessary for normal metabolic system activities.

Poultry meat is appropriate for quick and easy preparation; however, it recommends a variety of

combinations with diverse foodstuffs, thus, poultry meat can provide a frequent option for the meat consumers of this century's livelihood. According to López et al. (2019), poultry meat (leg part) is enriched with proteins called collagen, and hyaluronic acid, which have excellent biological functions, such as enhancement of cell proliferation, water-holding capacity, moisture absorption, retention, and are used as anti-aging in the skin. Compared with red meat, the major benefits of white chicken meat are lower caloric value and low amounts of saturated fat (Bernstein et al., 2010). Therefore, ingestion of white chicken meat is advisable for individuals who need to consume low-fat and to treat patients with cardiovascular problems (Metin and Orkide, 2017; Bingham and Riboli, 2004). Chicken meat contains the amino acid tryptophan, which affects brain cells, causing additional production of serotonin hormone, which helps to improve mood, relieve stress, and soothe (Marangoni et al., 2015).

CONCLUSION

Consumption of poultry meat as part of a plant-originated food is associated with a risk reduction of overweight and obesity, cardiovascular diseases, and type 2 diabetes mellitus. Additionally, white meat (poultry in particular) is considered moderately protective or neutral on cancer risk (gastrointestinal cancer, breast cancer). The importance of poultry meat for humans also has been recognized by different international institutions and societies, which consider this widely available, relatively cheap food to be specifically important in low-income countries, where it can help to meet the deficiency in important nutrients.

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Author's contribution

Sufian Abdo Jilo and Lenco Abdulhak Hasan have participated in the manuscript writing and edition of the paper. All authors read and approved the final version of the manuscript for publishing in the present journal.

Competing interests

The authors have declared that no competing interest exists.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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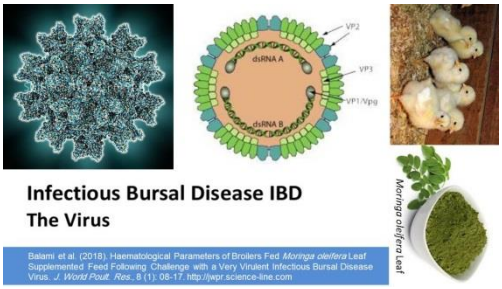

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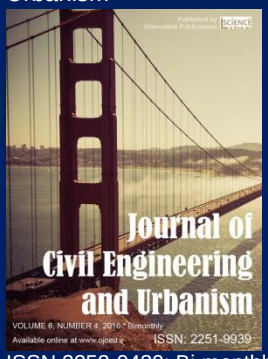

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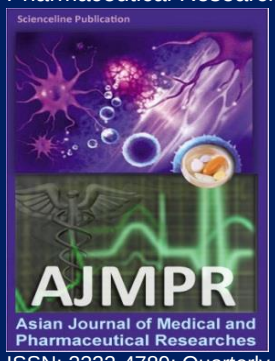

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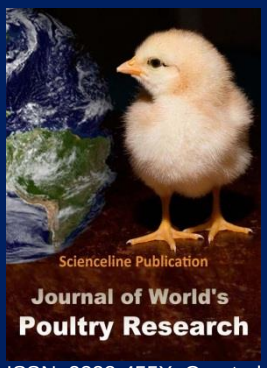

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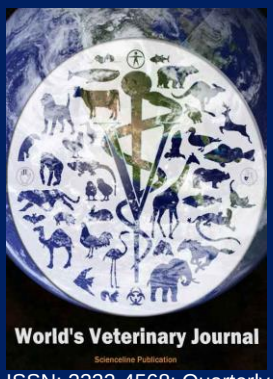

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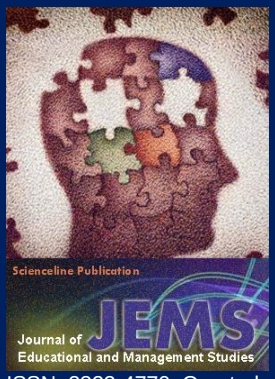

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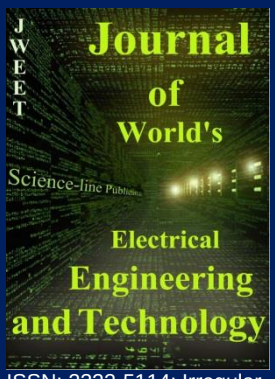

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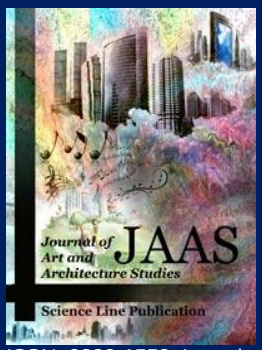

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