Feed utilisation, physiological and meat quality responses in Jumbo quail reared on apple pomace-containing diets

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DECLARATION

I, Allen Ngoanaoroele Matabane, hereby declare that this dissertation, in its entirety, has been written by myself and that the work contained herein is my own, except, where acknowledged by form of using a reference. This work has not been submitted for any other degree or academic qualification in any university other than the North-West University.

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GENERAL ABSTRACT

Quail (*Coturnix* sp.) farming has enormous potential to contribute to global food production and nutritional security by supplying high-quality animal protein to meet the nutritional demands of the rapidly growing human population. However, over-dependence on conventional feed ingredients such as maize (*Zea mays* L.) and soybean (*Glycine max*) when formulating quail diets is socially, economically, and environmentally unsustainable. Alternatively, the use of agro-industrial by-products such as apple (*Malus domestica* Borkh.) pomace, which are usually discarded to the detriment of the environment, could offer a sustainable long-term strategy to continuously supply bioactive compounds and essential nutrients for sustainable quail intensification while protecting the environment. Thus, this study assessed the effect of including graded levels of apple pomace powder (APP) on apparent nutrient digestibility, growth performance, blood parameters, carcass characteristics, internal organ sizes, and meat quality and composition in Jumbo quail. A total of 350, one-week-old unsexed Jumbo quail chicks (28.0 ± 0.828 g live weight), were evenly and randomly allotted to 35 replicate pens (experimental units). The birds were reared using five isonitrogenous and isoenergetic experimental diets (replicated seven times), which were formulated by including APP in a standard grower diet at a rate of 0 (AP0), 25 (AP25), 50 (AP50), 75 (AP75), and 100 g/kg (AP100). Daily feed intake and weekly liveweights were measured from week 1 to week 5. At five weeks of age, two female quail per pen were selected and used to measure apparent nutrient digestibility, while the rest of the birds were slaughtered at a nearby abattoir. Blood was collected during slaughter from two birds per experimental unit for measurement of serum biochemical and haematological parameters. Thereafter, the carcasses were eviscerated for measurement of carcass characteristics, internal organs, and meat quality parameters. Experimental diets significantly influenced organic matter digestibility (OMD) only, where quail on diet AP100 had higher (P <0.05) OMD (651.4 g/kg) than those on diet AP75 (542.1 g/kg). Linear and quadratic effects (P <0.05) were observed for

feed intake in week 2 in response to increasing dietary APP levels. In week 3, feeding incremental levels of dietary APP induced a significant quadratic effect for feed intake. Diet AP75 promoted the highest feed intake in weeks 2, 3, 4 and 5. Two-week-old Jumbo quail offered diet AP25 had higher (P <0.05) weight gain (30.41 g/bird) than those in the other treatment groups. However, in week 5, birds reared on diet AP75 (46.84 g/bird) had higher weight gain than all the other treatment groups. Significant linear decreases were observed for G:F in weeks 2, 3 and 4 in response to dietary APP levels. In week 5, G:F showed a significant quadratic response [$y = 0.240 (\pm 0.0167) + 0.002 (\pm 0.0009) x - 0.00002 (\pm 0.000008) x^2$; $R^2 = 0.151$; $P = 0.024$], from which an optimum inclusion level was calculated to be 50.0 g/kg APP. Diet AP25 promoted higher (P <0.05) serum calcium levels than diets AP75 and AP100. Quail reared on diet AP0 had the least alkaline phosphatase (ALKP) (166.1 U/L) content than those on diets AP75 and AP100. Diet AP0 produced heavier carcass weights than diet AP100. Diet AP0 promoted the heaviest proventriculus weight (0.778 % HCW) compared to all the other diets. One-hour yellowness (b^*_1) and 24-h (L^*_{24}) showed linear increases, while 24-h redness (a^*_{24}) and 24-h ($chroma_{24}$) linearly declined with increasing APP levels. Diet AP25 promoted higher pH_{24} values (5.59) than diets AP0, AP50, AP75 and AP100, which did not differ (P >0.05). Quail meat from birds fed diet AP75 had higher (P <0.05) crude protein (CP) (225.9 g/kg) than meat from quail fed with diets AP0, AP50 and AP100, whose CP content did not differ (P >0.05). Based on the quadratic response for G:F in week 5, an optimum APP inclusion of 50.0 g/kg was determined. It was concluded that dietary APP should be capped at 50 g/kg to maximize feed utilisation efficiency in Jumbo quail without compromising blood, carcass, and meat quality parameters.

Keywords: Agro-industrial waste, Health, Feed intake, Growth performance, Meat quality, Nutraceuticals, Nutrient digestibility

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LIST OF ABBREVIATIONS

AIBPs	:	Agro-industrial by-products
ADFD	:	Acid detergent fibre digestibility
AP	:	Apple pomace
APP	:	Apple pomace powder
CF	:	Crude fibre
CP	:	Crude protein
CPD	:	Crude protein digestibility
DM	:	Dry matter
DMD	:	Dry matter digestibility
EE	:	Ether extract
FA	:	Fatty acids
FI	:	Feed intake
G:F	:	Gain-to-feed ratio
GDP	:	Gross domestic product
NDFD	:	Neutral detergent fibre digestibility
OMD	:	Organic matter digestibility

1 CHAPTER ONE - GENERAL INTRODUCTION

1.1 Background

Globally, the poultry industry is regarded as one of the largest segments of animal food production. In South Africa, the poultry industry contributes over 50 billion rand per annum to the gross domestic product (GDP) of the country (Department of Trade and Industry, 2020) and is responsible for creating over hundred thousand jobs throughout its value chain (Ahaotu *et al*., 2019). The industry provides socio-economic and nutritional services to humans as a primary supplier of meat, eggs, and raw materials (feather & poultry litter) for various industries. These industries involve the production of feather meal and manure/fertilizers from feather and litter processing, respectively. According to the United States Department of Agriculture (USDA), the world chicken meat production increased from 99,027 metric tons (t) in 2019 to 100,026 metric t in 2020 in response to the nutritional demands of the rapidly growing human population (USDA, 2020). These numbers confirms that poultry meat has the highest production and consumption levels over beef, pork, and mutton (OECD/FAO *et al*., 2017). Indeed, the South African Poultry Association reported an annual per capita consumption of 38.93 kg for total poultry products (SAPA, 2020).

Chicken products are one of the major sources of animal protein for human consumption and due to the increasing demand for poultry products, efforts are currently being made to exploit other poultry species such as quail (*Coturnix* sp.) (Mahlake *et al*., 2021). Jumbo quail is a meat-type breed, which grows fast and reaches sexual maturity as early as six weeks of age (Marareni & Mnisi, 2020). The Jumbo quail is a larger and meatier version of the Japanese quail (*Coturnix coturnix japonica*) (Mbhele *et al*., 2019). Although quail farming is relatively a recent addition to the South African poultry industry, it already contributes towards household food and nutrition security as source of high-quality protein for human consumption (Mbhele *et al*., 2019). In addition, the quail has high resistance to diseases and can be used for meat production due to high meat-to-bone ratio (Mnisi & Mlambo, 2018). However, poor performance, disease outbreaks, high mortality rates and rising feed costs are persisting challenges affecting the development and sustainability of the poultry industry.

Nutritional strategies have been employed to maximize production through diet manipulation using various feed additives (Yusuf *et al*., 2016; Nduku *et al*., 2020). However, to sustainably intensify the production of the Jumbo quail, agro-waste by-products with functional bioactivities can be incorporated as feed ingredients in quail diets. One such waste by-product is apple (*Malus domestica* Borkh.) pomace, which is often disposed in barren lands/ landfills that are located closer to apple-juice manufacturing companies (Khan *et al*., 2015). Apple pomace (AP) is a by-product remaining from apple juice production, which has a potential as a feed material for animals owing to its low cost and high nutrient composition. The AP is a rich source of bioactive compounds such as carotenoids, phenolics, polysaccharides, phospholipids, and minerals, especially cobalt and iodine (Tkachuk, 2012; Barreira *et al*., 2019).

1.2 Problem statement

The major challenge in the long-term sustainability of intensive quail production is high feed cost, suboptimal performance, and poor meat quality (Rezaeipour *et al*., 2016). High feed cost is driven by the escalating prices of major conventional ingredients such as maize and soybean and the use of expensive feed additives to promote growth and control diseases (Tripati *et al*., 2017; Puvača *et al*., 2020). Therefore, agro-industrial wastes that have nutraceutical properties can be included in quail feeds to allow for sustainable intensification of the birds (Swain & Barbuddhe, 2008). The continuous disposal of agro-industrial waste in large volumes without following proper disposal channels poses a major environmental threat (Tayengwa & Mapiye, 2018; Kumanda *et al*., 2019). Accordingly, there has been rising concerns regarding the negative consequences caused by the disposal of AP in landfills (Perussello *et al*., 2017; Singha *et al*., 2018; Gowman *et al*., 2019).

The wanton disposal of AP in landfills interferes with plant growth and cause eutrophication in water streams. This is because the AP has high moisture content (>80%) (Gullion *et al*., 2008) and high biological oxidation demand and chemical oxidation demand values that are highly susceptible to microbial degradation (Lyu *et al*., 2020). On one hand, high moisture content in AP leads to great susceptibility to microbial decomposition, resulting in the production of greenhouse gases (GHGs) such as methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2). On the other hand, the dumping of this by-product can be a source of secondary pollution, such as the emission of foul smell due to microbial degradation. Further, landfilling of AP results in the contamination of underground water table due to nutrient or substance run off, especially in the rainy seasons (Taasoli & Kafilzadeh, 2008). Likewise, landfilling of AP creates breeding grounds for many vectors that can transmit diseases (Taasoli & Kafilzadeh, 2008). It is, therefore, imperative to find ways in which AP can be utilised without negatively affecting the environment.

One feasible strategy is to incorporate AP in quail diets as a nutraceutical source. However, the use of AP may also be limited by its low digestibility due to high fibre and lignin-to-cellulose ratio, and low crude protein and mineral contents (Correia *et al*., 2007). This indicates that the inclusion of apple pomace powder (APP) at higher dietary levels in quail diets would potentially reduce feed efficiency and growth performance while compromising the well-being of the birds (Aghili *et al*., 2019). As such, there is a need to establish the optimum inclusion level of APP in Jumbo quail diets. To this end, there are no studies that have investigated the effect of graded levels of APP on feed utilisation, growth performance, health, and meat quality attributes in Jumbo quail. This could be because the Jumbo quail is a newly developed breed in the poultry industry.

1.3 Justification

The use of AP as a nutraceutical in Jumbo quail diets could help maintain environmental equilibrium through waste reduction while providing essential nutrients and phytochemicals for sustainable quail production (Tayengwa & Mapiye, 2018; Gharehbagh *et al*., 2020). Indeed, according to Tayengwa & Mapiye (2018), using AP as a functional feedstuff in animal diets is a waste reduction strategy with the potential to improve food and nutrition security and environmental stewardship. Vieira *et al*. (2009) reported that AP contains growth-boosting bioactive substances such as polyphenols, which have antibacterial, antifungal, and antiviral properties that are responsible for many health benefits that are associated with a lower risk of chronic diseases.

In addition, AP has been used as a source of beneficial phytochemicals in poultry diets at 60 g/kg inclusion level with promising results such as high ileal viscosity, which can result in good digestibility of fibre, enlargement of digestive tissues and high growth performance in broilers (Colombino *et al*., 2020). Furthermore, the inclusion of APP in animal diets has been widely recommended due to sustainability aspects and their high level of readily fermentable carbohydrates (fructose, glucose, and sucrose) (Maslovarić *et al*., 2017). For instance, glucose and galactose metabolism can increase egg production and feed efficiency (Ayhan *et al*., 2009). In other studies, the inclusion of APP has been reported to boost immune status, reduce pathogenic microbial load in the digestive tract, and improve egg quality (Díaz-Plascencia *et al*., 2013; Aghili *et al*., 2019).

Moreover, AP is a rich source of polyphenols with antioxidant activities (Fanimo *et al*., 2003; Sudha *et al*., 2007), which is due to the presence of total flavonoids (0.45 - 1.19 mg/g), phenolics (4.22 - 8.67 mg/g), and total flavan-3-ols (2.27 - 9.51 mg/g) (Ćetković *et al*., 2008). Based on the findings of Ganai *et al*. (2006) and Islam *et al*. (2018), AP contains 19.34 - 20.66% crude fibre, 10 - 15% pectin, 7.31 - 8.53% crude protein, 2.6 - 3.33% ether extract, 46.4 - 49% neutral detergent fibre and 3.85 - 4.7% total ash. This shows that dried APP can be a potential source of nutrients and phytochemicals in poultry feed. Indeed, Zafar *et al*. (2005) indicated that dry APP can be used safely as an energy source in broiler diets to substitute 100 g/kg (w/w) maize without any negative effects on production. Moreover, Joshi *et al*. (2010) reported that feeding dried APP up to 150 g/kg to Cobb 500 broiler chickens had positive effects by increasing weight gain, while reducing mortality, and liver and kidney disorders, implying that it is a viable dietary by-product. Contrarily, Ayhan *et al*. (2009) suggested that dried APP can be used as a feed ingredient in broiler diets up to 50 g/kg. It is apparent that there are many varying results on the use of APP in poultry diets, which justifies the investigation of its feed value in Jumbo quail diets. Thus, establishing an optimum inclusion level for the Jumbo quail is critical given that most studies have focused on the use of APP in broiler chicken diets. This study, therefore, represents the first attempt to investigate the feed value of APP in Jumbo quail diets.

1.4 Aim and objectives

The aim of the study was to investigate the feed value of apple pomace powder in Jumbo quail diets. The following objectives guided the study:

i. To determine the effect of graded levels of apple pomace powder on apparent nutrient digestibility, growth performance, and haematology and serum biochemical parameters in Jumbo quail.

ii. To determine the effect of graded levels of apple pomace powder on carcass characteristics, sizes of internal organs, breast meat quality and stability, and meat composition of Jumbo quail.

1.5 Hypothesis

i. The first hypothesis tested whether the inclusion of incremental levels of apple pomace powder would improve apparent nutrient digestibility, growth performance, and haematological and serum biochemical parameters in Jumbo quail.

ii. The second hypothesis tested whether the inclusion of incremental levels of apple pomace powder would enhance carcass characteristics, internal organ development, breast meat quality and stability, and meat composition of Jumbo quail.

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2 CHAPTER TWO - LITERATURE REVIEW

2.1 Introduction

Over the last decade, global poultry consumption has increased more than all other meat types (USDA, 2020). This growth has been influenced by its relative affordability within the meat basket and has averaged to 2.5% per year over the past decade (BFAP, 2021). Apart from its low cost, white meat is regarded as a healthy commodity with no negative nutritional or religious connotations. As a result, poultry is expected to account for roughly half of the additional meat consumed globally in the next ten years (OECD-FAO, 2020). A large portion of this growth is anticipated in developing countries due to rapid population growth, increased urbanization, and rising incomes (Herrero *et al*., 2009). The growth in expenditure on animal source foods is expected to be higher among rural than urban consumers. This is because the highest human population growth is in rural areas (Colozza & Avendano, 2019). For example, in West Africa, expenditure on meat was projected to grow four times faster between 2010 and 2040 in rural areas than in urban areas (Zhou & Staatz, 2016). Among meat categories, poultry meat consumption in Sub-Saharan Africa increased from an annual average of 1.5 kg to 2.1 kg per capita between 2000 and 2017 (OECD-FAO, 2017). Projections of future demand for major meat products in Africa from 2010 to 2050 show that poultry meat is expected to grow by 813%, making it the fastest growing product followed by pork, beef, and mutton and chevon, which are projected to grow by 573%, 261%, and 162%, respectively (FAO, 2019).

The high demand for poultry products is also expected to drive production growth, however poultry productivity remains low in many African countries resulting in low product supply in the food chain. Production deficits are usually filled through international trade or imports, but there are rising concerns about food sovereignty (Amanor-boadu *et al*., 2016; Andam *et al*., 2017a). Likewise, the need to create jobs for Africa's growing youth population has the potential to shift policy towards rapid development of the poultry industry (Andam *et al*., 2017b). The effectiveness of such policies would be determined by consumer preferences for domestically produced poultry, among other factors.

Quail is a collective name for several genera of mid-sized birds generally placed in the order *Galliformes* (Mishra & Shugla, 2014). The name quail can be used in singular or plural forms. The rearing of quail birds has been characterized as economically sustainable and productive (Cardozo-Jiménez *et al*., 2008) because these birds grow fast (5 - 6 weeks maturity weight) (Mbhele *et al*., 2019) and are resistant to several numerous poultry diseases (Mnisi & Mlambo, 2018). Quail are the smallest domesticated poultry species, making them easy to manage and to accommodate even in landless production systems. The quail has relatively low floor area requirements, hence their production is regarded as a low-cost investment (Douglas, 2013). According to Mishra & Shugla (2014), about eight to ten adult quail birds can be reared in the same space required for one adult chicken. The Jumbo quail, which is the fastest growing and heavier version of the Japanese quail (*Coturnix coturnix japonica*), is regarded as a meat-purpose quail strain with a large body suitable for meat production and fair yield of eggs (Bughio *et al*., 2020). For sustainable quail intensification, alternative feed ingredients with high biological activities are required. Apple waste, a by-product from the apple juice processing industry, is a potential feed ingredient for quail owing to its low cost and high nutritional value (Alarcon-Rojo *et al*., 2019). Thus, the use of this non-conventional feed ingredient could be a valuable source of dietary nutrients (Shah *et al*., 2018), and a potential way of valorising agro-wastes and overcoming feed shortages. Although the pomace has been incorporated into poultry diets (Aghili *et al*., 2019; Colombino *et al*., 2020), there are currently no studies that have investigated its feed value in Jumbo quail diets.

2.2 Overview of the South African poultry industry

The South African poultry industry is made up of the day-old chick supply, the broiler, and layer businesses (SAPA, 2020). Within these three business branches, the South African Poultry Association (SAPA) represents both commercial and subsistence farmers (DAFF, 2017). Poultry production is the largest agricultural subsector in South Africa, producing nearly 19.6 million birds per week compared to all other animal subsectors (GAIN, 2020). Chicken meat was the most consumed in South Africa with a per capita consumption of 33 kg/year in 2019 (USDA, 2020; SAPA, 2020), down from 42 kg/year in 2018 (Berkhout, 2019). However, South Africa entered into a recession in the first quarter of 2020 due to the outbreak of the Coronavirus Disease 2019 (COVID-19) pandemic that caused a decline in gross domestic product (GDP), which was recorded at -2% (USDA, 2020). Consequently, chicken meat consumption declined in the country, but not as bad as compared to the per capita consumption of 17 kg/year for beef (USDA, 2020). The decrease in both production and consumption of poultry products was associated with low production of feed grains, which was because of low demand due to people losing jobs and the temporary closure of foreign imports or exports due to the global socio-economic crisis that was brought by COVID-19 (SAPA, 2020). This, therefore, necessitates the need to develop and diversify the South African poultry industry with avian bird species like the quail to complement chicken production.

2.3 Origin, distribution, and contribution of quail to the poultry industry

Quail farming started in Japan where wild quail birds were domesticated and bred in large numbers (Mondry, 2016). Kadani (2016) reported that the Japanese scientists first tamed the wild quail and publicized the ways to raise it as a domestic bird. To that effect, the Japanese quail (*Cortunix coturnix japonica*) has gained prominence in the commercial poultry subsector and is now widely distributed across many countries for commercial rearing, and meat and egg production (Egbeyale *et al*., 2013). Chivandi *et al*. (2020) and Akarikiya (2021) indicated that quail farming is now gaining popularity in Nigeria, Kenya, Zambia, and South Africa. However, the popularity of quail birds and production is still at an infancy stage in most developing African countries due to the lack of knowledge on quail farming. However, in some other parts of Africa (Ghana and Zimbabwe), quail farming is now common and established because of its role in alleviating protein malnutrition and food insecurity and also contributing to the economic empowerment of resource-poor farmers (Bakoji *et al*., 2013). Quail farming is practiced from subsistence to large-scale commercial operations (Monika *et al*., 2018).

Quail farming is one of the fastest growing poultry subsectors around the world (Puspamitra *et al*., 2014), and is currently gaining popularity in the South African poultry industry to complement chicken, turkey, and ostrich production. The contribution of quail to the human race is significant because they supply high quality meat and eggs, create job opportunities, and provide income to the farmers. Chivandi *et al*. (2020) added that quail meat and eggs are potential protein sources that can be used in the alleviation of protein-energy malnutrition and food and nutrition insecurity in South Africa. Furthermore, Mahlake *et al*. (2021) reported that the quail production has high return rate, low cost of investment, and nutraceutical benefits, which could explain why many poultry farmers are resorting to quail farming (Mondry, 2016). Quail hens raised on a high plane diet can lay up to 200 eggs in the first year of lay and have a life expectancy of two to two and half years (Onyewuchi *et al*., 2013; Nasar *et al*., 2016). Recent statistics have shown that quail production has increased considerably in South Africa primarily due to their desirable genetic potential such as fast growth rates, prolificacy, and early sexual maturity (Mnisi *et al*., 2022). The ability of the quail to reach market weight between five and six weeks means that producers do not have to wait for prolonged periods before selling their products. In European nations like Japan, France and Spain, the poultry industry is currently dominated by commercial quail production because of their immense abilities to survive various types of climatic and environmental conditions (Minvielle, 2004; Cavalcante *et al*., 2022).

2.4 Quail farming in South Africa

In South Africa, quail farming is still in its infancy stage and is slowly gaining the attention of many poultry producers (Mnisi *et al*., 2021). However, its rapid development is faced with a myriad of challenges such as the lack of adequate information on the birds' nutritional requirements under local conditions, lack of training for quail farmers, and excessive feed prices for major ingredients such as maize and soybean. The socio-economic benefits of quail farming in South Africa are still debatable. The rearing of quail has been practiced with minimal consideration to their general welfare, which has led to it receiving mixed reactions from both the general populace and government (Mazizi *et al*., 2022). This is due to the noise and the smell from the ammonia entrapped in the faecal matter they produce. Moreover, several factors such as feed costs, cold climate, predation, diseases, parasites such as enteric nematodes, and cannibalism restricts optimal quail production (Aboul-Ela *et al*., 1992). Farming quail also require knowledge on selecting the right breed. This is important because some breeds of quail have eating habits that results in feed wastages while some are aggressive, causing injuries to other quail birds, which affects performance (Randall & Bolla, 2008).

2.4.1 Quail production system

In South Africa, several farmers are already rearing quail birds for subsistence and commercial purposes, however, these farmers face challenges of high feed costs. As a result, these farmers have resorted to the use of various kinds of feeds such as chicken feeds because the game-bird diet, which is recommended for optimum quail production is more expensive. Nonetheless, the use of non-conventional feed resources such as agro-industrial by-products (AIBPs) is regarded as one of the strategies that can be used to reduce feed costs and deliver sustainable intensification. These by-products contain valuable nutrients and bioactive compounds that can benefit quail production and increase its contribution to food and nutrition security.

Ali *et al*. (2012) reported that quail production presents an opportunity to diversify and strengthen animal protein production as a as way to close the gap between demand and supply. Quail production could be a profitable business because it comes with great benefits of low labour and less capital requirements. Moreover, quail birds are resistant to several avian or poultry diseases, and thus eliminating the cost of vaccinations, antibiotics and other prophylactic treatments, which in turn promote the production of organic products (Chang *et al*., 2009; Bakoji *et al*., 2013). Indeed, the bioactive extracts derived from AP contain polyphenolic compounds (phenolic acid and flavonoids) and carotenoids, which have antibacterial and antifungal properties (Jridi *et al*., 2019; Abudayeh *et al*., 2019). The polyphenolic compounds found in AP have been shown to act as antimicrobial agents, by enriching beneficial gut bacteria (Gong & Yang, 2012), and inhibiting the growth of microorganisms depending on the structure and content of the hydroxyl group (Tripoli *et al*., 2007). These polyphenolic compounds may have positive effects on the oxidative and microbiological stability of quail products, thereby increasing their quality and shelf life (Tayengwa *et al*., 2020; Salami *et al*., 2020).

2.4.2 Quail nutritional requirements

Nutrient requirements of quail birds vary according to many factors such as environment, gender, age, and the stage of production, among others (Altine *et al*., 2016). Randall & Bolla (2008) stated that quail should have access to clean and fresh drinking water at all times because deprivation of water for more than 24 hours could result in death. However, there are several factors that affect water intake such as salt and protein content in the diet, and humidity and temperature levels. These factors must be kept constant in the quail house to achieve optimal production. Furthermore, Mishra & Shukla (2014) reported that quail consume 30 to 35 g per day, but feed should always be available to them. Currently, a standard ration for growing and breeding quail is not available in South Africa, which could be due to the fact that these birds are still cementing their place in the poultry industry as well as the lack of quail markets in South Africa. However, Randall & Bolla (2008) reported that a commercial game-bird diet can be used to attain optimum quail performance. The same authors reported that to achieve maximum production, the quail diet should contain approximately 250 g/kg crude protein, 12.6 MJ/kg of metabolizable energy, 4.5 g/kg phosphorus and 10 g/kg calcium for the first six weeks. Therefore, the inclusion of APP as a feed ingredient in quail diets would significantly meet the nutrient requirements of the quail because it contains high levels of soluble carbohydrates, proteins, amino acids and vitamins and minerals. However, due to the presence of anti-nutritional factors such as tannins, saponins, phytates and glucosinolates (Kara *et al*., 2018; Skinner *et al*., 2018), its dietary inclusion level that will promote optimal production without compromising the bird’s well-being require further investigation.

Table 2.1. Nutrient requirements (g/kg, unless otherwise stated) of quail in different feeding phases.

Nutrient	Starter	Grower	Finisher
[1]ME (Kcal/kg)	2800	2900	2750
Protein	270	240	240
Calcium	8.0	6.0	8.0
Phosphorus	3.0	3.0	3.0
Vitamin A (IU)	8000	8000	8000
Vitamin D3 (IU)	1200	1200	1200
Riboflavin	0.006	0.006	0.006
Lysine	13.0	13.0	12.0
Methionine	4.8	4.5	4.5
Methionine + Cysteine	7.5	7.0	7.0
Valine	9.5	9.2	9.0
Threonine	10.2	7.4	7.0
Tryptophan	2.2	1.9	6.8
Phenylalanine	9.6	7.9	7.7

Source: Chakrabarti *et al*. (2014), [1]ME = metabolizable energy.

2.5 The use of agro-industrial by-products as alternative feed sources

Agro-industrial by-products (AIBPs) are defined as material that is produced during the processing, manufacturing, distribution, transportation, or consumption of food from agro-industries (Westendorf *et al*., 1996). Research has shown that AIBPs consist of nutrients and bioactive substances that could benefit animal nutrition in terms of growth and health (Ristanovic *et al*., 2009). The use of agro-wastes such as AP could serve as viable alternatives to conventional ingredients in animal diets (Álvarez-Fuentes *et al*., 2012). The use of agro-wastes could reduce the already existing feed-food competitions because they have no direct food value for humans (Bampidis & Robinson, 2006). Further, the incorporation of agro-wastes in quail feeds could contribute positively to environmental, economic, and social sustainability.

According to Fang *et al*. (2016), agro-wastes such as apple, grape, and pomegranate pomaces, among others, have been used in pigs (Chedea *et al*., 2019), broiler (Kumanda *et al*., 2019) and quail (Fróes *et al*., 2018; Mnisi *et al*., 2021) diets as a source of nutraceuticals. However, AIBPs that could be of nutritional use and financial value in animal production remain poorly developed or unexploited (Agbo & Prah, 2014). This could be due to scanty information on their nutritive value and/or valorisation strategies to improve their feed value in animal feeds. Moreover, the utility of these by-products is restricted by the presence of secondary metabolites (tannins, gossypol, lectins, phytic acid, oxalic acid and glucosinolates) (Francis *et al*., 2001), as well as their high fibre and fat contents (Bedford & Schulze, 1998). If used appropriately, agro-wastes can overcome the high demand for conventional nutrient sources and close the gap on feed shortages. It is critical, therefore, to investigate the effects of these by-products on quail growth performance, health, and meat quality.

2.5.1 The use of agro-wastes as a strategy to achieve environmental sustainability

The world's population has risen from 3.7 billion in 1970 to 8 billion in the current year 2022 (StatsSA, 2022). It is also predicted to reach 9.8 billion by 2050 and 11 billion by 2100, respectively (Koops & van Leeuwen, 2017). Consequently, there is a daunting challenge to achieve food security in the coming years (Godfray *et al.*, 2010). To fulfil the increasing nutritional demands for the growing human population, there has been a significant rise in livestock and crop production, which has further contributed towards the generation of agro-wastes (Tripati *et al.*, 2017). During the last century, Asia and Africa have not only experienced rapid population, as well as economic growth, but also a rise in agro-waste production capacities (Wang *et al.*, 2018).

Moreover, South Africa produces a bulk of solid waste every year, in which AIBPs remain on top with ~80 000 million t (GAIN, 2020). This translates to large amounts of agro-wastes that are disposed in landfills without proper disposal methods. Consequently, this leads to the generation of greenhouse gases (GHGs) such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4), which have a high warming potential (Searchinger *et al.*, 2008; Kaab *et al.*, 2019).

The major categories of AIBPs are crop residues (leaf litter, seed pods, stalks, stems, straws, husks, weeds), livestock wastes (urine, dung, wash water, residual milk, waste feed), poultry waste (spilled feed, feathers, droppings, bedding material), slaughterhouse waste (blood, hair, hides, flesh, bones), agro-industrial waste (bagasse, molasses, peels (orange, potato, cassava) pulps (orange, apple, mango, guava, pomegranate, pineapple, papaya, tomato), oil-seed cakes (palm kernel cake, groundnut, soybean, mustard, coconut) and aquaculture wastes (uneaten feed, faecal waste) (Tripati *et al.*, 2017; Seidavi *et al.*, 2019; Duque-Acevedo *et al.*, 2020), which have all sparked public concerns due to land, soil, water and air pollution.

Nonetheless, these agro-wastes require viable management strategies to make full use of them. One waste management strategy is to valorise them into value-added products to improve farmer's livelihood and create job opportunities for youth and, in turn, achieve the United Nation's sustainable development goals (Agamuthu, 2009; Bracco *et al*., 2018). For instance, AP can be used as livestock feed and can also be converted from low-value material into high-quality edible product (FAO, 2019; Ominski *et al*., 2021), in the form of silage or through various enzyme application techniques. Furthermore, to improve the nutritive value of AP, a variety of methods can be used to reduce or eliminate the presence of toxic secondary compounds such as tannins. Tannin-binding agents (polyethylene glycol, polyvinylpyrrolidone, polyvinyl polypyrrolidone, acetic acid, and sodium hydroxide), enzymes, heating, water-soaking, drying, wood ash, chopping, urea, and solid-state fermentation are among these methods (Ben Salem *et al*., 2000; Silanikove *et al*., 2001; Makkar, 2003).

2.5.2 The use of agro-wastes in animal diets

Generally, feed costs constitute over 70% of the total cost of production (González-Alvarado *et al*., 2010). An increase in feed cost translates to a reduction in profit margins, which negatively affect farm productivity. Poultry diets are largely composed of maize and soybean meal as sources of energy and protein, respectively. These two nutrient sources are the most preferred broiler and layer feed ingredients owing to their essential feeding value (Stein *et al*., 2009). The demand for these ingredients is rapidly growing to cater animal production and human consumption because in some countries maize and soybean is used as staple food for humans (Khojely *et al*., 2018). This is ironical because the availability of these conventional feed ingredients remains a major problem in low- and middle-income countries, which places a heavy economic burden to poultry producers. There is, therefore, a clear competition for maize and soybean between humans and animals (Capper, 2013). The demand for these resources is further exacerbated by the growing population, which is projected to reach 9.6

billion by 2050 (Bremner, 2012). Consequently, prices for these ingredients are expected to continue to increase on the global markets (McGlone, 2013). Indeed, the greatest constraint to poultry productivity for resource-poor farmers in South Africa is the shortage of feeds, which can be alleviated through better utilization of locally available resources, such as agro-waste by-products. The waste by-products can be included in animal feeds to supply much-needed dietary nutrients and bioactive compounds.

Agro-wastes are available in substantial quantities from fruit/vegetable processing and oil production industries, and often contain valuable nutrients that can benefit animal production (De Evan *et al*., 2019). However, to reduce production waste in landfills, waste by-products such as AP can be utilized as a natural source of dietary nutrients and antioxidants in poultry nutrition. According to Goñí *et al*. (2007), a balanced diet with increased level of polyphenols from grape pomace can prevent lipid oxidation in broiler diets that contain high levels of unsaturated fatty acids. Moreover, the presence of polyphenols in poultry diets could also play a significant role in regulating the microbial activity of the gastrointestinal tract, and thus the physiological morphology and functionality of the intestines (Fotschki *et al*., 2019). Recently, different type of fruit pomaces has been studied as novel feeds in poultry nutrition. Some studies have shown that dried fruit pomaces from berries added to diets increased oxidative stability of turkey meat without impairing growth performances (Juśkiewicz *et al*., 2015; 2017). Jankowski *et al*. (2016) observed beneficial effects on blood antioxidant parameters in turkey fed with diets supplemented with apple, strawberry or blackcurrant pomaces in terms of increased vitamin C concentration, integral antioxidant capacity of lipophilic and hydrophilic substances and decreased lipid peroxide levels.

2.6 Production trends of apple fruits

According to FAOSTAT (2021), the global production of apples is estimated at 184,925 hectograms per hectare. The deciduous fruit industry in South Africa is well-established and primarily aimed at supplying fresh apples, pears, peaches, nectarines, plums and apricots to the export market (Tharaga, 2016). South Africa is the second largest apple (*Malus domestica* Borkh.) exporter in the southern hemisphere, after Chile, and sixth in the world. About 44% of apples produced in South Africa are exported, with the Far East and Asia being the largest importers, accounting for about 32%, followed by the African market (31%), the United Kingdom (17%), the Middle East (7%), Europe (6%), Russia (4%), and Indian Ocean Islands (3%) as shown in Figure 2.1. In South Africa, about 25% of the apples produced are sold in the local markets while 31% are processed into various products (Hortgro, 2019). During the 2018/19 season, apples contributed approximately 28.2% (R5.8 billion) of the total gross value for deciduous fruits (R10.28 billion) in South Africa (Hortgro, 2019).

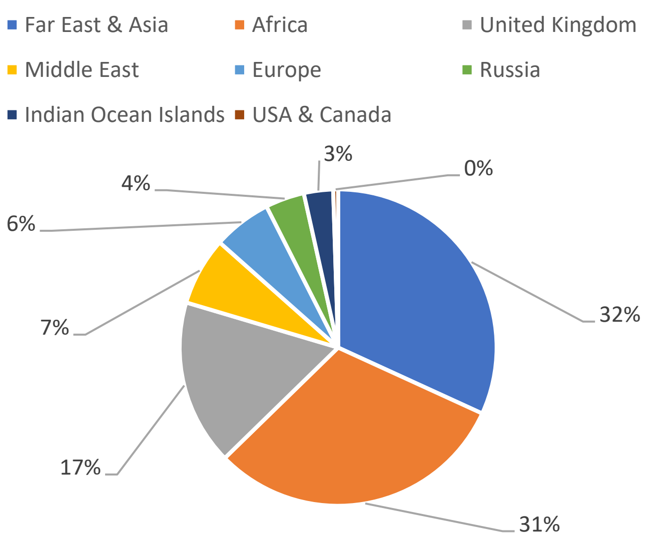

Figure 2.1. South African apple exports per market destination (Source: Hortgro, 2019).

2.7.1 Different apple cultivars in South Africa

Apples are currently the major deciduous fruit planted in South Africa and represent more than a third of the total deciduous fruit area (Hortgro, 2018). In South Africa, most apples are grown in the Western Cape followed by the Eastern Cape provinces. According to Thomas *et al*. (2020), Western Cape has the highest production of apple and pear pomace (154 tons/week) compared to the Northern and Eastern Cape provinces (40 and 46 tons/week, respectively). Indeed, the Western Cape province accounts for more than half of the country's apple production and the main producing areas are in the Koue Bokkeveld near Ceres, and in Groenland (Elgin/ Grabouw/ Villersdorp/ Vyeboom). Smaller production areas are found along the Orange River in the Free State province, and in Mpumalanga and Limpopo provinces. Annual reports show that the area under cultivation has steadily increased from 22,167 ha in 2012 to 24,971 ha in 2019 with over 35 million trees planted (Hortgro, 2019). In some key production areas in the Western Cape, irrigation is vital to meet the water requirements of the trees given that most rain falls during the winter months (May to August) and very little precipitation is received during the fruit growing season (September to April).

The main apple cultivars that are commercially grown in South Africa are Golden delicious, Royal gala, Granny smith, Pink lady and Top red (DAFF, 2012). In 2020, it was recorded that Golden delicious recorded 22% (4737 ha) of the area planted, which was followed by Royal gala at 17% (2720 ha) and Granny smith at 14% (4782 ha) (GAIN, 2022). Pink lady and Top red followed with 12% (1363 ha) and 10% (1925 ha), respectively (Chen *et al*., 2012). These five cultivars dominate apple production in South Africa and account for more than 70% of the planted area (GAIN, 2022). Other cultivars that have been growing steadily include Fuji, Cripps red, Braeburn big bucks, Kanzi and others as shown in Figure 2.2. The cultivars of choice are mainly determined by consumer preferences and demand in South Africa's export markets (USDA, 2020). Harvest for South African apples typically begins at the end of January and runs through to June, with peak harvest times falling between February and April. However, apples are available throughout the year in South Africa due to storage in temperature and air-controlled cold rooms.

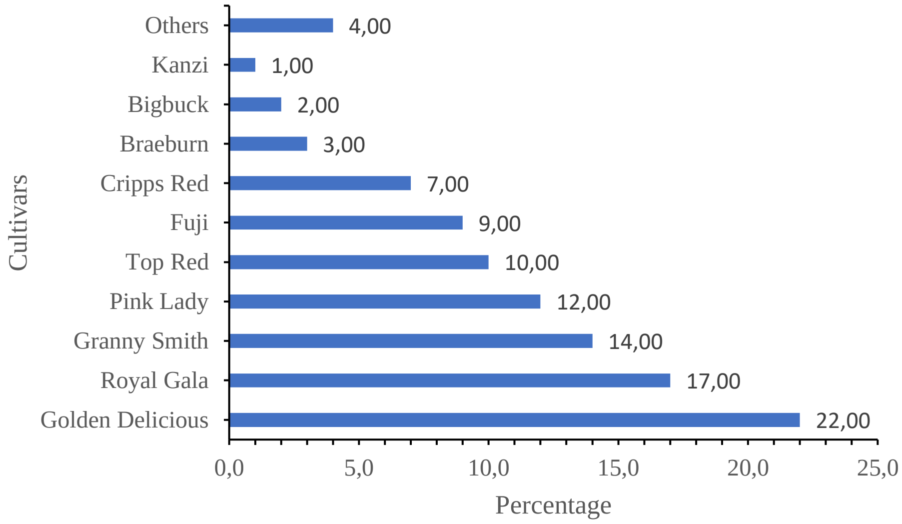

Figure 2.2. Area planted to different cultivars in South Africa in proportion to the total area under apple cultivation in 2019 (Source: Hortgro, 2019).

2.7.2 Generation of apple pomace

According to Molinuevo-Salces *et al*. (2020), the production of apples in the world is approximately 54.2 million tons per year. Around 26% of this production is processed in the apple industry for producing different products such as juice, jelly or cider (Dhillon *et al*., 2013). The solid waste biomass produced after generating the different apple products is called apple pomace (AP) and it accounts for approximately 25% of the total processed biomass. Therefore, the processing of apples generates tons of AP every year. According to Pachapur *et al*. (2015), AP takes 25 - 30% weight of the whole fruit. An enormous amount of fruit waste is disposed into landfills or barren land while some is incinerated every year, resulting in additional losses to industries and a further increase of greenhouse gas emissions. This happens when AP deposited into a landfill undergoes anaerobic (without oxygen) decomposition stage where methane-producing bacteria (methanogens) begin to decompose the waste and generate methane (Shurson, 2020). Only 20% of AP is utilized for animal feed due to its rapid spoilage in wet conditions (Dhillon *et al*., 2013). Therefore, the utilisation of this by-product as a source of animal feed would be of great economic importance to help food manufacturing plants reduce disposal costs while reducing the negative environmental impact that comes with the disposal of this by-product (DAFF, 2017; Arowolo & He, 2018). As an intervention by the government, a memorandum of understanding can be signed by the state and the agro-fruit industry to allow the collection and distribution of the waste to resource-poor farmers. In addition, farmers can preserve the by-product by sun-drying and storing it for the dry season. Therefore, more research is required to refine these waste biomasses for adoption by the feed manufacturing industries.

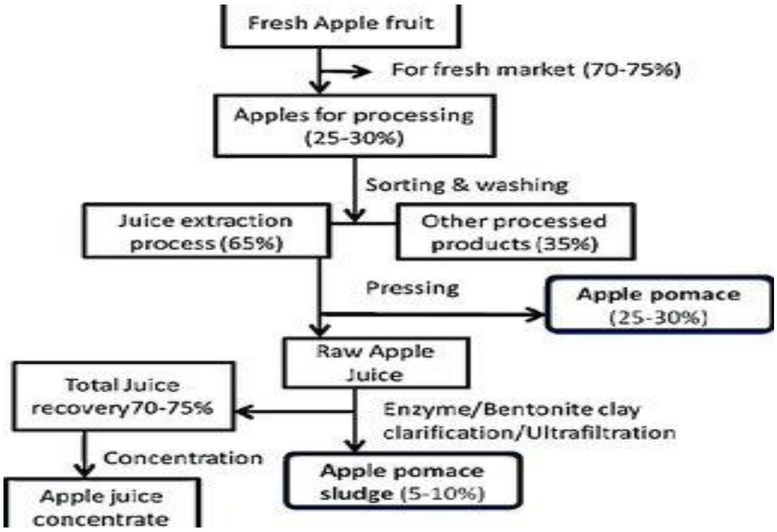

Figure 2.3. Processing of apples to generate apple pomace (Source: Dhillon *et al*., 2013).

2.7.3 Regulatory restrictions and safety usage of the apple pomace

The incorporation of agro-waste in animal diets is limited by regulatory policies in South Africa. This is because these by-products are sometimes contaminated by soil-borne pathogens when disposed into landfills, which may lead to development of soil-borne diseases such as acute gastroenteritis and clostridial myonecrosis (Nair, 2021). Furthermore, the Feed Acts in South Africa strongly restricts the use of several unprocessed by-products such as AP in animal diets (Thomas *et al*., 2020) without prior processing. Thus, the processing of the waste by-products could assist in minimizing the chances of transmitting pathogens such as *Clostridium perfringes* and *Aspergillus fumigatus* (Nair, 2021) that may survive during the contamination of the waste biomass. These by-products are usually utilized by resource-poor farmers around the agro-manufacturing industries. However, these resource-poor farmers are advised and urged to follow the procedures and protocols of the South African Animal Feed Act (Act 36, 1947), which strictly prohibits the use of agro-industrial by-products as feed ingredients or as the sole feed without prior processing. The by-products can be processed in the form of drying, pelleting and silage-making. However, some of these techniques can be expensive for resource-poor farmers (Ajila *et al*., 2015). As a result, these farmers can process their by-products through sun-drying (He *et al*., 2018), which offers a good solution to preserve them for future use. The processing of the waste by-products assists to minimize the chances of transmitting pathogens that may occur through contamination, which could adversely affect the well-being of the animals. Thus, the processing and use of the agro-wastes as potential feed ingredients in quail diets will mitigate the disposal labour and environmental pollutions (Wang *et al*., 2014).

2.8 Dietary effects of apple pomace in poultry nutrition

In the last two decades, the use of AP has gained popularity due to its nutraceutical properties that could positively impact animal production. The ability of AP to improve growth performance is primarily determined by its form and the amount incorporated into the diet (Beermann *et al*., 2021). Furthermore, AP has been shown to have antioxidant activity that can be used to enrich animal products with health-promoting bioactive compounds for consumers (Hygreeva *et al*., 2014; Jankowski *et al*., 2016). For example, AP promotes the population of beneficial gut microflora through its prebiotic mode of action (Beermann *et al*., 2021; Kithama *et al*., 2021). However, the utilization of AP may vary based on the components of dietary fibre present in the supplied feed as well as the function of the gut for a specific animal species. This is because dietary fibre present in AP is rich in the arabinoxylan, a non-starch polysaccharide (NSP) (Jha & Mishra, 2021). Therefore, the composition of the NSP portion of AP usually influence its utilisation, especially by poultry species. In addition, the fibre's solubility and water holding capacity determine its viscosity, and fermentability impacts lower gut utilization and health. High viscosity is reported to decrease the rate of endogenous enzyme diffusion into the digesta, which reduces nutrient digestion (Sadeghi *et al*., 2020). Thus, it is important to determine the maximum tolerance inclusion level of AP that would improve its utilization in Jumbo quail without compromising their health status.

2.8.1 Effects of apple pomace-containing diets on growth performance

Currently, there are inconsistencies in literature regarding the impact of AP on growth performance of poultry. Likewise, various studies have, to this end, reported different optimum inclusion levels, which indicates that the utilisation of the AP is influenced by many factors. According to Akhlaghi *et al*. (2014), the inclusion of dried AP up to 250 g/kg did not affect body weight (BW) of breeder roosters, but significantly reduced fertility, hatchability, and increase embryonic mortality. Furthermore, Bhat *et al*. (2000) and Ayhan *et al*. (2009) reported that inclusion level of 100 g/kg AP was found to be optimal in maintaining broiler chicken BW, while an inclusion level of 150 g/kg resulted in reduced BW and increased FCR. In addition, Akhlaghi *et al*. (2014) concluded that long-term administration of dried AP at 50 g/kg improved reproductive performances in Cobb 500 breeder roosters. Moreover, Juśkiewicz *et al*. (2015) reported that feeding 50 g/kg of AP to turkey did not affect growth performance and post-slaughter parameters. Furthermore, feeding 50 g/kg of fermented AP significantly improved the efficiency of feed conversion from 55.4 to 92.1% on growing pigs (Ajila *et al*., 2015). Furthermore, fruit by-products such as AP contain a high concentration of pectin and/or methoxyl, which may impair nutrient absorption by increasing goblet cells in the small intestine (Erinle & Adiwole, 2022). The primary cause of these inconsistencies could be because of varying nutrient and polyphenol profiles, harvesting site of the apples, age, gender, and type of species used in the studies.

2.8.2 Effect of apple pomace on carcass characteristics and meat quality

Carcass yield and carcass cut weights are essential meat industry characteristics since they are used to grade meat products and have a direct impact on market prices. The quality of meat and quail performance are influenced by the type of feed given to the birds (Muchenje *et al*., 2009; Mahlake *et al*, 2021). Apple pomace has gained interest for use in animal nutrition because of its high nutritional value and bioactive substances, which may be beneficial in attaining optimal performance and product quality in quail (Beermann *et al*., 2021). For instance, Heidarisafar *et al*. (2016), stated that adding 10 g/kg of AP in diets increased the weight of the gizzard and small intestine from Cobb 500 broiler chickens. There is currently limited literature on the use of AP on carcass characteristics and organ development in poultry.

The preference and acceptance of any meat product is determined by consumers based on the carcass' visual appearance, and compositional and sensory characteristics (Lawrie & Ledward, 2006). According to Muchenje *et al*. (2009), meat quality is determined by physical, chemical, sensory, technological, nutritional, and culinary properties that consumers find desirable. The pH and colour of meat have a significant impact on its quality attributes, with meat pH being used as a predictor of tenderness, shelf life, water holding capacity, and drip loss (Traore *et al*., 2012; England *et al*., 2014). The extent and rate of muscle pH drop is determined by the amount of reserved muscle glycogen that would be converted to lactic acid during anaerobic respiration post-slaughter (King, 2006). Post-mortem biochemical processes influence the colour of muscle foods. As the proportion of metmyoglobin increases, the meat colour turns to brown.

Changes in colour during chilling storage have been observed by other authors. Mazur-Kuśnirek *et al*. (2019) reported a lower yellowness (*b**) value in the breast muscles of broilers fed with polyphenols-enriched diets extracted from AP. Jiang *et al*. (2007) also found an increased pH and an increased *L** value of meat colour in broiler fed with soybean isoflavone rich in polyphenols. Tenderness appears to be the most important sensory characteristic of meat and a predominant quality determinant. Moreover, water holding capacity (WHC) is one of the most important indicators of meat quality because it indicates the ability of fresh meat to retain its own water during cutting, heating, grinding, and pressing, and during transport, storage, and cooking. The water released can be described as drip, purge, weep, exudate or cook loss (Hughes *et al*., 2014). In a study conducted by Pieszka *et al*. (2017), it was reported that meat quality was significantly improved by reducing total cholesterol by 12.4% and red colour value when 100 g/kg of dried AP was fed to finisher pigs. This points to the strong antioxidant activity of AP in meat products of animals reared on diets containing phytogenics such as AP (Guil-Guerrero *et al*., 2016). Moreover, other studies have found that the polyphenols responsible for AP antioxidant activity are still present in the pomace and apple peel (Gazalli *et al*., 2014; Shanmugam *et al*., 2017). In addition, some antioxidative vitamins and minerals found in AP plays a role in preventing structural damage to muscle tissue of meat products (Hercberg *et al*., 2004). Because of this, AP has already been used in the formulation of several meat products without affecting overall palatability (Rather *et al*., 2015). However, its feed value remains unknown for the Jumbo quail.

2.8.3 Effects of apple pomace containing diets on poultry health

Whole blood count is a common method for monitoring the pathophysiological and general health status of birds in response to nutritional and environmental factors, infectious diseases, and stress. Moreover, erythrocyte, haemoglobin and MCH levels are indicators of how much oxygen is delivered to body tissues (Washington & Hoosier, 2012). Blood tests can be used to detect the presence of many metabolites and other elements in animal bodies since blood plays an important role in an organism's physiological, nutritional, and pathological conditions (Aderemi, 2004; Doyle, 2006). Blood parameters in poultry production can provide critical information for disease diagnosis and prognosis (Olafedehan *et al*., 2010). The blood profile and health status of farm animals can be affected by feed ingredients. Haematological components such as red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration are important in monitoring feed toxicity (Oyawoye & Ogunkunle, 2004).

According to Heidarisafar *et al*. (2016), the addition of 100 g/kg AP to diets increased the weight of chicken gizzard and small intestine. Dietary treatments containing 50 and 100 g/kg apple peel waste increased lipid and high-density lipoprotein (HDL) levels while decreasing low-density lipoprotein (LDL) and malondialdehyde levels in blood serum. The increase in HDL is good for consumers because it absorbs cholesterol in blood and carries it back in the liver, and therefore reduces the risks of heart diseases and strokes while LDL however, it is considered bad to consumers because it raises risks for heart diseases and strokes (Froyen, 2021). In addition, a study by Nobakht (2013) indicated that feeding (0, 25, 50, and 75 g/kg) of AP to laying hens resulted in the highest levels of lymphocytes when hens were reared on the 50 g/kg treatment compared to the other treatment groups. These results shows that the inclusion of dietary AP has the potential to affect blood parameters. Thus, it is important to establish an optimum inclusion level so as not to compromise the health status of the Jumbo quail.

2.9 Summary

Quail farming is a reliable source of high-quality protein for human consumption and has the potential to alleviate food and nutrition insecurity in developing countries. Finding alternative functional feed ingredients with bioactive substances such as AP could improve quail performance and product quality. However, APP contains high levels of fibre and other antinutrients (saponins, tannins, glucosinolates and phytates) that may limit its utilization in quail diets, especially at higher inclusion levels. It is, therefore, imperative to determine the optimum inclusion level of this ingredient in order to safely use it as a rich source of natural antioxidants and other bioactive compounds in Jumbo quail-based diets. To this end, no studies have investigated the effect of APP on apparent nutrient digestibility, growth performance, blood indices, carcass characteristics, internal organs, and breast meat quality and meat composition of Jumbo quail. It is, therefore, imperative to investigate the maximum inclusion level of APP in Jumbo quail diets in order to close the existing knowledge gaps in this literature.

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3 CHAPTER THREE - APPARENT NUTRIENT DIGESTIBILITY, GROWTH PERFORMANCE AND BLOOD INDICES IN JUMBO QUAIL REARED ON APPLE POMACE-CONTAINING DIETS

Abstract

The study evaluated the effect of graded levels of apple pomace powder (APP) on apparent nutrient digestibility, growth performance, and blood parameters in Jumbo quail. A total of 350, one-week-old mixed-gender Jumbo quail chicks (28.0 ± 0.828 g live weight) were randomly and evenly distributed into 35 pens (10 birds/pen), which were replicated seven times per experimental diet. Five isonitrogenous and isoenergetic experimental diets were formulated by including APP at 0 (AP0), 25 (AP25), 50 (AP50), 75 (AP75) and 100 g/kg (AP100) in a standard grower diet. Average weekly feed intake (AWFI) and average weekly body weight gain (ABWG) were recorded and used to calculate average weekly gain-to-feed ratio (G:F). At the end of the growth trial (five weeks of age), two female quail were left in each replicate pen (experimental unit) to allow for a five-day digestibility trial. The rest of the birds (n = 280) were slaughtered after stunning and blood samples were immediately collected for haematological and serum biochemical analyses. Experimental diets significantly influenced organic matter digestibility (OMD), where quail fed diet AP100 had higher (P <0.05) OMD (651.4 g/kg) compared to those fed diet AP75 (542.1 g/kg). There were significant linear and quadratic effects for feed intake in week 2 in response to increasing dietary APP levels. In week 3, incremental levels of dietary APP resulted in a significant quadratic trend for feed intake [R^2 = 0.160; P = 0.024]. Two-week-old quail offered diet AP25 had higher weight gain (30.41 g/bird) than those offered diets AP50, AP75 and AP100. Five-week-old birds reared on diet AP100 had lower weight gain (34.39 g/bird) than those reared on diet AP75 (46.84 g/bird). Two-week-old quail fed with diets AP0 and AP25 had the highest G:F, followed by those fed with AP50 and AP75 (P >0.05) and the lowest was from those fed with diet AP100. There were linear decreases for G:F in week 2 [R^2 = 0.799; *P* = 0.0001], week 3 [R^2 = 0.609; *P* = 0.0001] and week 4 [R^2 = 0.649; *P* = 0.0001] in response to dietary AP levels. In week 5, G:F showed a positive quadratic response [R^2 = 0.151; *P* = 0.024], which was optimised at 50 g/kg APP level. A linear increase was observed for serum phosphorus [R^2 = 0.188; *P* = 0.018], calcium [R^2 = 0.188; *P* = 0.026] and lymphocytes [R^2 = 0.303; *P* = 0.001], but a linear decrease was observed for platelet count [R^2 = 0.180; *P* = 0.017] as dietary APP levels increased. Heterophils showed negative linear [R^2 = 0.266; *P* = 0.042] and quadratic trends [R^2 = 0.266; *P* = 0.012]. It was concluded that including APP at rate of 50.0 g/kg maximizes gain-to-feed ratio and does not compromise physiological parameters in adult Jumbo quail.

Keywords: Apple pomace, Growth performance, Haematology, Nutrient digestibility, Serum biochemistry

3.1 Introduction

Quail products (meat and eggs) are important sources of animal protein for human consumption (Mazizi *et al*., 2022), which can be used to alleviate protein-energy malnutrition and food security risks in rural South Africa (Mnisi *et al*., 2022). Quail products have high nutritional value that can provide significant amounts of amino acids such as lysine, threonine, methionine, cysteine, and tryptophan, as well as omega-6 and omega-3 polyunsaturated fatty acids (Dowarah, 2013; Pereira & Vicente, 2013; Chepkemoi *et al*., 2017). These polyunsaturated fatty acids lower the risk of developing obesity and metabolic diseases by improving both glucose (White *et al*., 2015) and lipid metabolism (Bellenger *et al*., 2019) and by alleviating liver lipid storage through downregulation of lipogenic genes (Marangoni *et al*., 2015). Therefore, quail farming has been encouraged in developing countries to help address socioeconomic concerns and hunger (Chadoka, 2017). These rise in socioeconomic challenges has led to the large-scale intensification of quail to bridge protein deficit (Mazizi *et al*., 2019; Oladimeji *et al*., 2020). In addition, the rapid adoption of Jumbo quail production in Sub-Saharan Africa (SSA) can complement chicken production.

Despite the advantages of quail farming, high feed costs have a negative impact on sustainable production of these birds. High feed cost, which is driven by the overreliance on conventional feed ingredients (Owen & Dike, 2013), is a major constraint to quail production (Mnisi & Mlambo, 2018). Thus, to reduce the overreliance on conventional feed sources such as maize and soyabean meal (SBM), it is critical to incorporate agro-waste by-products such as the apple pomace (AP) to supply much needed dietary nutrients and bioactive compounds.

Several agro-waste by-products have been recently evaluated in poultry diets and positive results were recorded (Aghili *et al*., 2019; Colombino *et al*., 2020; Erinle & Adewole, 2022). Furthermore, Pirmohammadi *et al*. (2006) and Kara *et al*. (2018) reported that dietary substitution of maize with 100 g/kg of dried AP did not adversely affect growth performance in Cobb 500 broiler chickens. Similarly, Juśkiewicz *et al.* (2015) reported that feeding 50 g/kg of dried AP to turkey did not negatively affect growth performance and post-slaughter parameters in young turkeys. In another study, feeding 50 g/kg of fermented AP reported improved feed conversion efficiency from 55.4 to 92.1% in growing pigs (Ajila *et al*., 2015). Although there is vast literature on the use of AP in diets of various animal species, its feed value has not been investigated for the Jumbo quail.

The use of APP in animal nutrition has its own limitations, which include the presence of anti-nutritional factors such as saponins, tannins, glucosinolates and phytates, and high fibre levels (Kara *et al*., 2018; Skinner *et al*., 2018). This indicates that the inclusion of APP at higher dietary levels in quail diets would reduce feed efficiency and growth performance while compromising the well-being of the birds (Aghili *et al*., 2019). As such, there is a need to establish the optimum inclusion level of APP in Jumbo quail diets. Therefore, this study was designed to investigate the optimum inclusion level of APP as a nutraceutical in Jumbo quail using apparent nutrient digestibility, growth performance, and haematology and serum biochemistry as response indicators. It was hypothesized that the inclusion of APP would improve nutrient utilisation, growth performance, and blood parameters of the birds Jumbo quail.

3.2 Material and methods

3.2.1 Animal rights statement

The rearing and slaughter procedures of the quail were approved by the Animal Research Ethics Committee for Animal Production studies of the North-West University (approval no. NWU-00807-21-A5) according to established guidelines for use and care of research animals.

3.2.2 Research site

The study was conducted between October and November 2021 at the North-West University Research Farm (Molelwane). The study area is located in the semi-arid region of the North-West province (South Africa), with the following geographical coordinates: 25°40.459′ S and 26°10.563′ E. The study site is situated at an altitude of 1226 m above sea level and receives an average annual rainfall ranging from 300 to 600 mm. The site experiences summer from August to March with temperatures ranging from 17 to 37°C and winter from May to July with temperatures ranging between 2 and 20°C, respectively.

3.2.3 Ingredient sources

Fresh, wet apple (*Malus domestica* Borkh.) pomace (containing a mixture of yellowish, red and green apples) was donated by Elgin Fruit Juices (Pty) Ltd, which is situated in Grabouw, Western Cape province of South Africa. The apple trees are growing at an altitude of 326 m above sea level, located at 34.15215° S, 19.01143° E, where they receive an average annual temperature of 15.5°C, with an average rainfall of 694 mm per annum, which is mostly received in winter than summer. Upon arrival, the AP was sun-dried for a period of 5 days (constant weight) and thereafter packaged in paper bags for milling. The AP was ground (2 mm; Polymix PX-MFC 90 D, Kinematica AG, Malters, Switzerland) to produce the apple pomace powder (APP). The other feed ingredients were bought from Nutroteq and Simplegrow Agric Services (PTY) LTD (Centurion, Gauteng, South Africa).

3.2.4 Proximate analysis

Prior to blending with other ingredients, the APP was analysed for laboratory dry matter (DM), crude protein (CP), crude fibre (CF), crude fat and minerals (calcium, phosphorus, sodium, chloride and potassium). For DM determination, approximately 1 g of AP sample was placed into a pre-weighed crucible and placed in an oven set at 105°C for 12 hours. After 12 hours, samples were removed from the oven, placed in desiccators to cool and then weighed. The loss of weight was measured as moisture content and DM was calculated as the difference between initial sample weight and moisture content weight. Organic matter content was determined by incinerating the dried APP samples in a muffle furnace set at 600°C for 12 hours. The crucibles were thereafter removed, placed in desiccators to cool, and then reweighed. The loss in weight was measured as OM content. The ANKOM^{2000} Fibre Analyser (ANKOM Technology, New York) was used to determine crude fibre by using the crude fibre acid solution (0.255 N) and the crude fibre base solution (0.313 N). In addition, the randall submersion method (RSM) (AOAC 2005; method no. 2005.05) was used to determined crude fat. Metabolizable energy was determined using the near infrared reflectance spectroscopy (NIRs) models (SpectraStar XL, Unity Scientific, Australia) as described by Mnisi and Mlambo (2017). Total nitrogen was determined by the standard macro-Kjeldahl method (AOAC 1995, method no. 984. 13) and then converted to CP by multiplying N content by the factor 6.25. The amino acids profile and mineral contents (calcium (Ca), phosphorus (P), sodium (Na), chloride (Cl) and potassium (K)) were analysed following the guidelines by the Agri-Laboratory Association of Southern Africa (AgriLASA. 1998). Total soluble phenolics (TSPh) were determined following the Folin–Ciocalteau method (Makkar, 2003). Absorbance was recorded at 725 nm wavelength using a spectrophotometer (T60 UV-Visible spectrophotometer, PG Instruments Limited, Lutterworth, UK) and expressed as tannic acid equivalent (g TAE/kg DM). Soluble condensed tannins (SCT) were determined using the Butanol-HCl method (Porter *et al*., 1986) and absorbance was measured at 550 nm wavelength using the spectrophotometer described above.

3.2.5 Diet formulation

The dietary treatments were formulated to meet the daily nutritional requirements of growing quail birds (National Research Council, 1994). The experimental diets, in a mash form, were formulated by treating a standard grower diet with graded levels (0, 25, 50, 75 and 100 g/kg) of APP using a nutritional software. The diets were formulated to have the same energy (isocaloric) and protein (isonitrogenous) levels. The nutritional compositions of the test diets were determined according to the methods described in Section 3.2.4.

Table 3.1. Ingredient composition (g/kg, *as is* basis) of the experimental diets offered to Jumbo quail.

	[1]Diets				
Ingredients	AP0	AP25	AP50	AP75	AP100
Apple pomace	0	25	50	75	100
Yellow maize (8.5%)	437.3	524.3	469.3	334	440.8
Extruded full fat soya	0	0	260	250	250
Soya oil cake (44%)	350.6	379.2	106.6	22.6	163.1
Sunflower oil cake (38%)	68.4	0	81.2	243.3	14.7
DL-Methionine	1.4	1.4	0.9	0	0.6
Soya oil crude	65.1	30.4	1.6	45.6	0
Limestone (slow solubility)	0	24.1	23.4	22.4	23.7
Salt (Fine)	1.6	1.7	1.7	1.5	1.8
Sodium bicarbonate	15.4	10.9	2.4	2.5	2.2
Custom premix	3	3	3	3	3
[2]MDCP	56.1	0	0	0	0
Lysine-HCL	1	0	0	0	0

[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]MDCP = mono-dicalcium phosphate.

Table 3.2. Nutritional composition of the experimental diets (g/kg *as is* basis, unless stated otherwise).

[2]Components	[1]Diets				
	AP0	AP25	AP50	AP75	AP100
Dry matter	922.1	941.6	935.6	944.2	967.8
Organic matter	861.0	890.3	882.8	887.0	907.4
Ash	51.14	51.29	52.86	57.14	50.43
Arginine	15.45	15.28	16.44	18.96	17.27
Histidine	5.76	6.05	6.42	6.96	7.07
Isoleucine	9.8	10.29	10.94	11.85	12.21
Lysine	12.6	12.6	12.72	13	14.56
Methionine	5.0	5.0	5.0	5.0	5.0
T.S.A.A.	8.55	8.59	8.84	9.3	8.99
Threonine	8.49	8.88	9.39	10.2	10.36
Tryptophan	2.74	2.85	3.04	3.36	3.4
Valine	10.93	11.22	12.04	13.64	13.08
Linoleic acid	41.08	26.26	36.68	54.97	33.7
Crude protein	230.0	230.0	230.0	230.0	230.0
Crude fibre	37.46	39.67	41.75	43.86	44.50
Crude fat	87.89	65.83	71.54	108.02	75.77
ME (MJ/kg)	12.50	12.50	12.50	12.50	12.50
Calcium	10.1	10.1	10.0	10.0	10.0
Chloride	1.4	1.45	1.45	1.45	1.45
Potassium	9.52	9.64	8.79	8.22	9.0
Sodium	5.0	3.81	1.5	1.5	1.5
Total phosphorus	14.18	3.54	4.23	5.26	4.41
TSPh (g TAE/kg)	19.36	22.38	25.73	21.38	34.64
SCT (AU)	0.083	0.077	0.169	0.392	0.583

[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder and AP100 = a standard grower diet with 100 g/kg of apple pomace powder. [2]Components: ME = metabolizable energy, T.S.A.A = total sulphur amino acids, TSPh = total soluble phenolics, SCT = soluble condensed tannins.

3.2.6 Experimental design, birds and growth trial

A total of 350, four-days-old unsexed Jumbo quail chicks were purchased from T/A Golden Quail Farm (Randfontein, Gauteng, South Africa) and transported by road to Molelwane Research Farm. Upon arrival, the chicks were randomly allocated into 35 pens (100 cm long × 60 cm wide × 30 cm high), with each pen containing 10 birds, simulating a completely randomized design. The chicks were offered a stress pack (containing vitamins and electrolytes) administered through drinking water for the first three days. In addition, the birds were acclimatized to the pens and adapted to the experimental diets until one week of age. The dietary treatments were randomly allocated to the 35 pens (experimental units), which were replicated seven times per treatment group. The pens were made of wire-mesh floor and polythene plastics were used as bedding. Measurements commenced from one week to five weeks of age. Fresh, clean water and the diets were offered *ad libitum* during the 35-day feeding trial. Rearing was done under natural lighting from morning (06h00) to evening (18h00).

3.2.7 Feed intake and growth performance

At one week of age, initial live weights (28.0 ± 0.828 g) of the birds were taken and subsequently weighed weekly per pen until five weeks of age to determine average weekly body weight gain (ABWG). Average weekly feed intake (AWFI) per bird was measured as the difference between the weights of feed offered and feed refused and divided by the total number of quail in a pen. The data for ABWG and AWFI were computed and used to determine average weekly gain-to-feed ratio (G:F) using the following formula:

$$G{:}F = \frac{Weight\ gain\ (g)}{feed\ consumed\ (g)}$$

3.2.8 Slaughter procedure, blood collection and analysis

At day 35 of age, two randomly selected birds per pen were left for the measurement of apparent nutrient digestibility. The rest of the birds were transported by road to a locally registered poultry abattoir for slaughter. At the abattoir, the birds were allowed to rest for a minimum of 2 hours before they were electrical stunned and then slaughtered humanely by cutting the jugular vein with a sharp knife. While bleeding, 4 ml of blood, from two randomly selected birds per pen, was collected and immediately transferred into two sets of tubes using 5 ml syringes and 23 *g* needles. The two sets of tubes differed according to lid colours with purple-top tubes containing ethylene-diamanie-tetra-acetic acid (EDTA), an anti-coagulant for haematological analysis and red-top tubes without any anticoagulant for serum biochemical analysis. The tubes containing the blood were stored in a cooler bag and the haematological samples were analysed within 24 h after collection (Washington & Hoosier, 2012). Haematological parameters (haematocrit, platelet counts, heterophils, lymphocytes, monocytes, and white cell count (WCC)) were determined using an automated LaserCyte Haematology Analyser (IDEXX Laboratories, (Pty) Ltd. Johannesburg, South Africa). Serum biochemical parameters (total protein, urea, creatinine, albumin, globulin, amylase, calcium, phosphorus, choline, lipase, albumin/globulin ratio (ALB/GLOB), total bilirubin (TBIL), serum symmetric dimethylarginine (SDMA), alanine transaminase (ALT) and alkaline phosphatase (ALKP)) were analysed using an automated Vet Test Chemistry Analyser (IDEXX Laboratories (Pty) Ltd. Johannesburg, South Africa).

3.2.9 Apparent nutrient digestibility

At day 35 of age, two quail from each pen were randomly selected for measurement of apparent nutrient digestibility. The birds were given a three-day adaptation period prior to a five-day faecal collection period. Feed offered and excreta were collected in each replicate pen. The excreta were collected once daily, packaged, and frozen at -15ºC to prevent fermentation of samples pending proximate analysis. At the end of the collection period, feed and faecal samples were weighed and homogenized. Apparent digestibility values of DM, OM, CP, CF, EE, NDF and ADF were calculated as described by Bashar *et al*. (2010) using the following formula:

$$Apparent\ nutrient\ digestibility\ (\%) = \frac{nutrient\ intake - fecal\ nutrient}{nutrient\ intake} \times 100$$

3.2.10 Data analysis

Data (feed intake, apparent nutrient digestibility, growth performance, serum biochemistry and haematology) were subjected to normality tests using the normal option in Proc Univariate statement (SAS® 9.4, 2010). Average weekly feed intake, average weekly body weight gain and average weekly gain-to feed ratio data were analysed using the repeated measures analysis in the General Linear Model procedure (GLM PROC) of SAS® 9.4 (2010). The following statistical linear model was employed:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk}$$

Where, Y_{ijk} = response variables, μ = population mean, D_i = effect of diets, W_j = effect of week (quail age), $(D \times W)_{ij}$ = effect of interaction between diets and week, E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed.

Data on feed intake, apparent nutrient digestibility, growth performance, serum biochemistry and haematology were further evaluated for linear and quadratic effects using polynomial contrast. A response surface regression analysis (PROC RSREG; SAS, 2010) was employed to estimate the optimum dietary inclusion level of APP using the following quadratic equation:

$$y = ax^2 + bx + c$$

Where: *y* = dependent variable; *a* and *b* are the coefficients of the quadratic equation; and *c* is dietary APP levels. The *x* value for optimal response was determined as: $\frac{-b}{2a}$.

One-way analysis of variance (ANOVA) was also used to account for dietary differences on feed intake, apparent nutrient digestibility, body weight gain, gain-to-feed ratio, and serum biochemical and haematological parameters data by means of using the GLM procedure of SAS (2010). The linear statistical model employed was as follows:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where, Yij = dependent variable, μ = population mean, Di = effect of diets, and Eij = random error associated with observation *ij*, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P < 0.05$ and least squares means were compared using the probability of difference.

3.3 Results

3.3.1 Nutrient digestibility and growth performance

Table 3.3 shows that there were neither linear nor quadratic effects (*P* >0.05) for dry matter digestibility (DMD), organic matter digestibility (OMD), neutral detergent fibre digestibility (NDFD), acid detergent fibre digestibility (ADFD) and crude protein digestibility (CPD) in response to increasing levels of dietary APP. Experimental diets had no effect (*P* >0.05) on DMD, NDFD, ADFD and CPD, but significantly influenced OMD. Quail fed diet AP100 had higher (*P* <0.05) OMD (651.4 g/kg) compared to those fed diet AP75 (542.1 g/kg). However, birds fed diet AP0 had similar (*P* >0.05) OMD as the other treatment groups.

Table 3.3. Apparent nutrient digestibility (g/kg DM, unless stated otherwise) of apple pomace-containing diets in Jumbo quail.

	[1]Diets						Significance		
[2]Parameters	AP0	AP25	AP50	AP75	AP100	[3]SEM	*P* value	Linear	Quadratic
DMD (g/kg)	536.8	584.2	589.9	503.0	623.8	29.24	0.053	0.349	0.382
OMD	559.8[ab]	620.5[ab]	640.5[ab]	542.1[a]	651.4[b]	26.86	0.025	0.289	0.929
NDFD	469.2	499.8	517.9	399.9	475.9	32.77	0.139	0.426	0.981
ADFD	487.3	501.0	525.5	415.0	494.9	31.94	0.179	0.499	0.723
CPD	736.6	757.1	741.3	775.6	715.9	19.41	0.279	0.718	0.194

[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]Parameters: DMD = dry matter digestibility, OMD = organic matter digestibility, NDFD = neutral detergent fibre digestibility, ADFD = acid detergent fibre digestibility, and CPD = crude protein digestibility.
[3]SEM = standard error of the mean.

Repeated measures analysis showed significant week × diet interaction effects on ABWG (*P* = 0.003), AWFI (*P* <0.000) and G:F (*P* <0.000). Table 3.4 shows that, in week 2, including different levels of APP induced significant linear and quadratic effects on feed intake [y = 63.9 (±1.531) + 0.29 (±0.073) x – 0.002 (±0.001) x^2; R^2 = 0.515; *P* = 0.015]. In week 3, incremental levels of dietary APP resulted in a significant quadratic trend for feed intake [y = 96.8 (±2.58) + 0.255 (±0.122) x – 0.003 (±0.001) x^2; R^2 = 0.160; *P* = 0.024]. Neither linear nor quadratic effects (*P* >0.05) were recorded for feed intake in weeks 4 and 5.

Two-week old Jumbo quail birds fed with diet AP0 had the least AWFI (63.46 g/bird) compared to those fed with the other diets, which did not differ (*P* >0.05). In week 3, diet AP75 (106.72 g/bird) promoted higher (*P* >0.05) AWFI than diets AP0, AP50 and AP100, which were statistically similar (*P* >0.05). However, three-week-old birds reared on diet AP75 had similar (*P* >0.05) AWFI with those reared on diet AP25. Furthermore, three-week-old birds reared on diet AP0 had similar (*P* >0.05) AWFI with birds reared on diets AP25, AP50 and AP100. In week 4, diet AP75 (133.8 g/bird) promoted higher AWFI than diets AP25 and AP100, which did not differ (*P* >0.05). However, the control diet promoted similar (*P* >0.05) feed intake as the other treatment groups. Diet AP75 promoted higher (161.9 g/bird) feed intake in five-week-old birds than diets AP0, AP25 and AP100, whose feed intake was similar (*P* >0.05). However, diet AP50 promoted similar (*P* >0.05) feed intake as the other treatment groups.

Table 3.4. Average weekly feed intake (g/bird) of Jumbo quail reared on diets containing graded levels of apple pomace powder.

	[1]Diets						Significance		
	AP0	AP25	AP50	AP75	AP100	[2]SEM	*P* value	Linear	Quadratic
Week 2	63.46[a]	71.52[b]	71.93[b]	76.27[b]	74.57[b]	1.610	0.0001	0.0001	0.015
Week 3	96.94[ab]	103.06[bc]	96.38[ab]	106.72[c]	92.16[a]	2.258	0.001	0.501	0.024
Week 4	130.5[ab]	122.6[a]	127.9[ab]	133.8[b]	123.0[a]	2.028	0.002	0.650	0.594
Week 5	148.7[a]	141.7[a]	151.9[ab]	161.9[b]	149.5[a]	2.676	0.0001	0.051	0.396

[a,b,c] In a row, means with common superscripts do not differ ($P > 0.05$).
[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]SEM: standard error of the mean.

Table 3.5 shows that weight gain linearly decreased in week 2 [$y = 30.219\ (\pm 0.9031) - 0.046\ (0.0433)\ x$; $R^2 = 0.567$; $P = 0.000$], week 3 [$y = 42.579\ (\pm 1.2318) - 0.056\ (\pm 0.0577)\ x$; $R^2 = 0.528$; $P = 0.000$] and week 4 [$y = 48.073\ (\pm 1.5078) - 0.191\ (\pm 0.0706)\ x$; $R^2 = 0.527$; $P = 0.000$] as dietary APP levels increased. However, no significant quadratic trends were observed in weeks 2, 3 and 4. On the other hand, weight gain in week 5 quadratically responded [$y = 34.6\ (\pm 2.89) + 0.331\ (\pm 0.137)\ x - 0.003\ (\pm 0.005)\ x^2$; $R^2 = 0.156$; $P = 0.046$] with increasing dietary APP levels.

The GLM results showed that two-week-old quail offered diet AP25 had higher ($P < 0.05$) weight gain (30.41 g/bird) than those offered diets AP50, AP75 and AP100. However, two-week-old quail fed with diet AP25 had similar ($P > 0.05$) weight gain as those fed with the control treatment AP0. In week 3, quail reared on AP100 had the least (31.47 g/bird) weight gain compared to all the other dietary treatments. Birds reared on diet AP0 had similar ($P > 0.05$)

weight gain with those reared on diets AP25 and AP75, whilst those reared on diet AP25 were also statistically similar (P >0.05) to those reared on diets AP50. In week 4, quail fed with diet AP0 had the highest (P <0.05) weight gain (49.63 g/bird) followed by those fed with diet AP50 (41.45 g/bird), and the lowest weight gain was on the AP100 group (34.98 g/bird). Moreover, four-week-old birds fed with diet AP25 had similar weight gain to those fed with diet AP75 and AP50. The AP100 group also had similar (P >0.05) weight gain as the AP75 group. In week 5, birds reared on diet AP100 had lower (P <0.05) weight gain (34.39 g/bird) than those reared on diet AP75 (46.84 g/bird). Nonetheless, the control treatment (AP0) promoted similar (P >0.05) weight gain as the other treatment groups in week 5.

Table 3.5. Average weekly body weight gain (g/bird) of Jumbo quail reared on diets containing graded levels of apple pomace powder.

	[1]Diets						Significance		
	AP0	AP25	AP50	AP75	AP100	[2]SEM	*P* value	Linear	Quadratic
Week 2	29.64^{bc}	30.41^{c}	26.02^{ab}	24.93^{a}	22.64^{a}	0.921	0.000	0.000	0.437
Week 3	43.34^{c}	39.91^{bc}	37.17^{b}	39.07^{bc}	31.47^{a}	1.151	0.000	0.000	0.454
Week 4	49.63^{c}	40.25^{ab}	41.45^{b}	39.74^{ab}	34.98^{a}	1.426	0.000	0.000	0.299
Week 5	35.36^{ab}	40.61^{ab}	39.74^{ab}	46.84^{b}	34.39^{a}	2.936	0.041	0.661	0.023

^{a,b,c} In a row, means with common superscripts do not differ (P >0.05).
[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]SEM: standard error of the mean.

Table 3.6 shows that there were significant linear decreases for G:F in week 2 [y = 0.47 (±0.011) – 0.003 (±0.0005) x; R^2 = 0.799; P = 0.0001], week 3 [y = 0.439 (±0.009) – 0.002 (±0.0004) x; R^2 = 0.609; P = 0.0001] and week 4 [y = 0.38 (±0.009) – 0.002 (±0.0004) x; R^2 = 0.649; P = 0.0001] in response to dietary APP levels. However, there were no quadratic effects

(P >0.05) observed for G:F in weeks 2, 3 and 4. In week 5, G:F showed a significant quadratic response [y = 0.240 (±0.017) + 0.002 (±0.001) x – 0.00002 (±0.00001) x^2; R^2 = 0.151; P = 0.024], from which an optimum inclusion level was calculated to be 50.0 g/kg APP. There were no linear effects (P >0.05) observed for G:F in week 5 as dietary APP levels increased.

Two-week-old quail fed with AP0 and AP25 had the highest G:F followed by those fed with AP50 and AP75 (P >0.05) and the lowest was from those fed with AP100. In week 3, quail offered diet AP0 had the highest (P <0.05) G:F (0.447) and the lowest G:F was observed for those reared on diet AP100 (0.341). In week 4, the highest (P <0.05) G:F was observed on quail fed with diet AP0 followed by AP50 and AP75, and the lowest was observed from those fed diet AP100. Dietary treatment had no significant effect on G:F in week 5.

Table 3.6. Average weekly gain-to-feed ratio (g:g) in Jumbo quail reared on diets containing graded levels of apple pomace powder.

	[1]Diets						Significance		
	AP0	AP25	AP50	AP75	AP100	[2]SEM	*P* value	Linear	Quadratic
Week 2	0.467^{c}	0.428^{c}	0.362^{b}	0.326^{ab}	0.304^{a}	0.012	0.0001	0.0001	0.163
Week 3	0.447^{c}	0.388^{b}	0.387^{b}	0.366^{ab}	0.341^{a}	0.010	0.0001	0.0001	0.218
Week 4	0.381^{c}	0.328^{b}	0.324^{b}	0.297^{ab}	0.284^{a}	0.009	0.0001	0.0001	0.125
Week 5	0.238	0.286	0.262	0.288	0.230	0.018	0.093	0.824	0.024

[a,b,c] In a row, means with common superscripts do not differ (P >0.05).
[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]SEM: standard error of the mean.

3.3.2 Serum biochemical parameters

Table 3.7 shows that there were no significant linear and quadratic effects for serum biochemical parameters in Jumbo quail, except for phosphorus [y = 0.027 (±0.016) x + 3.69 (±0.333); R^2 = 0.188; P = 0.018] and calcium [y = 0.003 (±0.005) x + 2.074 (±0.098); R^2 = 0.188; P = 0.026], which linearly increased (P <0.05) in response to incremental levels of dietary APP.

Dietary influences (P <0.05) were observed on serum calcium, alkaline phosphatase (ALKP), lipase and choline. Diet AP25 promoted higher (P <0.05) calcium levels than diets AP75 and AP100, which did not differ (P >0.05). However, diet AP25 induced similar calcium levels (P >0.05) as diets AP0 and AP50. Quail reared on the control treatment (AP0) had the least ALKP (166.1 U/L) content compared to those on diets AP75 and AP100, which did not differ (P >0.05). Birds fed with diet AP50 produced similar ALKP levels as those on the other treatment groups. Birds reared on diet AP25 (3.86 mmol/L) showed higher choline levels than those on diets AP75 and AP100, which did not differ (P >0.05). However, diet AP25 promoted similar (P >0.05) choline levels as diets AP0 and AP50. Diet AP0 promoted higher lipase content (191.7 U/L) compared to the control diet. However, diet AP0 produced similar (P >0.05) lipase content with diets AP25, AP50 and AP100, which did not differ (P >0.05).

Table 3.7. Serum biochemical parameters of Jumbo quail reared on diets containing graded levels of apple pomace powder.

[2]Parameters	[1]Diets					[3]SEM	Significance		
	AP0	AP25	AP50	AP75	AP100		*P* value	Linear	Quadratic
SDMA (μg/dL)	30.00	35.29	23.73	30.14	35.00	4.393	0.352	0.734	0.316
Creatinine (μmol/L)	9.00	8.56	8.96	11.41	8.64	1.169	0.407	0.576	0.562
Urea (mmol/L)	0.94	1.04	1.31	1.25	1.03	0.151	0.382	0.415	0.093
Phosphorus (mmol/L)	3.59	4.48	4.64	4.66	4.91	0.362	0.127	0.018	0.293
Calcium (mmol/L)	2.01^{ab}	2.24^{b}	2.08^{ab}	1.83^{a}	1.83^{a}	0.101	0.035	0.026	0.170
Total protein (g/L)	31.21	31.43	32.00	33.29	35.79	1.873	0.418	0.503	0.445
Albumin (g/L)	15.14	14.36	15.79	14.14	13.36	0.820	0.292	0.155	0.325
Globulin (g/L)	21.43	21.57	21.93	21.57	22.14	1.625	0.998	0.776	0.981
ALB/GLOB	0.74	0.79	1.00	0.73	0.72	0.133	0.548	0.839	0.236
ALT (U/L)	31.86	37.93	32.29	39.29	42.21	2.906	0.177	0.092	0.123
ALKP (U/L)	166.1^{a}	175.4^{ab}	234.0^{abc}	261.4^{bc}	320.4^{c}	23.316	0.000	0.924	0.062
TBIL (μmol/L)	2.43	3.00	2.39	2.99	2.50	0.312	0.439	0.899	0.454
Choline (mmol/L)	3.03^{ab}	3.86^{b}	2.81^{ab}	2.71^{a}	2.70^{a}	0.267	0.025	0.065	0.540
Amylase (U/L)	138.8	214.9	164.6	180.9	199.4	20.271	0.104	0.215	0.556
Lipase (U/L)	115.6^{a}	170.1^{ab}	186.8^{ab}	125.9^{ab}	191.7^{b}	19.212	0.022	0.120	0.497

[a,b,c] In a row, means with common superscripts do not differ ($P > 0.05$).
[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]Parameters: SDMA = serum symmetric dimethylarginine, ALB/GLOB = albumin/globulin ratio, ALT = alanine transaminase, ALKP = alkaline phosphatase, TBIL = total bilirubin.
[3]SEM: standard error of the mean.

3.3.3 Haematological parameters

Table 3.8 shows that there were no linear nor quadratic effects (P >0.05) for haematocrits, WCC, heterophils and monocytes in response to incremental levels of dietary APP. Platelet count linearly declined [y = 21.47 (±3.92) – 0.008 (±0.186) x; R^2 = 0.180; P = 0.017] with increasing dietary APP levels. Heterophils showed negative linear and quadratic trends [y = 0.002 (±0.001) x^2 – 0.135 (±0.069) x + 11.06 (±1.461); R^2 = 0.266; P = 0.012] as dietary APP levels increased. A linear increase was recorded for lymphocytes [y = 0.071 (±0.157) x + 36.45 (±3.317); R^2 = 0.303; P = 0.001] in response to increasing dietary APP levels.

Jumbo quail birds offered diet AP100 produced higher lymphocytes (53.79%) than those on diet AP0 (35.71%). However, both diets AP0 and AP100 promoted similar (P >0.05) lymphocytes as diets AP25, AP50 and AP75. Birds on diet AP100 produced higher (P >0.05) heterophils (15.43 $\times 10^9$/L) than those on diet AP25 (8.11 $\times 10^9$/L). However, birds on both diets AP25 and AP100 had similar heterophils content as those on diets AP0, AP50 and AP75.

Table 3.8. Haematological parameters of Jumbo quail reared on diets containing graded levels of apple pomace powder.

Parameters	[1]Diets					[3]SEM	Significance		
	AP0	AP25	AP50	AP75	AP100		*P* value	Linear	Quadratic
Haematocrits (%)	43.53	41.86	45.71	46.24	47.07	1.528	0.126	0.089	0.529
[2]WCC ($\times10^9$/L)	21.67	21.54	22.53	22.77	23.63	2.284	0.966	0.543	0.875
Platelets ($\times10^9$/L)	22.63	18.73	27.91	27.82	34.74	4.209	0.109	0.017	0.434
Heterophils (%)	49.91	37.89	39.93	41.74	43.09	4.181	0.332	0.124	0.564
Lymphocytes (%)	35.71[a]	40.06[ab]	42.97[ab]	44.87[ab]	53.79[b]	3.603	0.019	0.001	0.541
Heterophils ($\times10^9$/L)	11.21[ab]	8.11[a]	9.81[ab]	10.06[ab]	15.43[b]	1.578	0.032	0.042	0.012
Monocytes ($\times10^9$/L)	1.03	1.14	1.46	1.69	1.81	0.408	0.734	0.766	0.163

[a,b,] In a row, means with common superscripts do not differ (*P* >0.05).
[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]WCC = white cell count.
[3]SEM: standard error of the mean.

3.4 Discussion

3.4.1 Nutrient digestibility and growth performance

Nutrient digestibility refers to the portion of dietary nutrients that are absorbed and assimilated into an animal's body as ingested feed passes through the gastrointestinal tract (Egbu *et al*., 2022). In this study, dietary inclusion of graded levels of APP in Jumbo quail diets did not affect DM, NDF, ADF and CP digestibility values. Nonetheless, the inclusion of increasing APP levels up to 50 g/kg in quail diets tended to increase protein digestibility, which corroborates the reports of Olayinka *et al*. (2022), who noted an increase in protein digestibility in Japanese quail reared on garlic meal-containing diets. The numerically lower CPD at 100 g/kg of APP could be due to the negative relationship that exists between polyphenols and dietary protein. Indeed, polyphenols bind to proteins via the interaction of their reactive hydroxyl groups with the protein's carbonyl group, which reduces protein digestibility (Lund, 2021). Dietary inclusion of APP influenced OM digestibility only. The OMD increased with increasing APP levels, which indicates high availability of organic nutrients for digestion and absorption by the birds. Numerically, all the other nutrients from this study decreased with increasing APP levels, which demonstrates that higher levels of APP could compromise feed utilisation efficiency. The decrease in the digestibility of the other nutrients could be induced by the extra dietary fibre in the APP-containing diets. Abioye *et al.* (2006) reported similar observation in broiler chickens, where birds fed kolanut (*Cola acuminata*) husk-based diets showed a decrease in feed utilisation efficiency than birds in the control group. The reduced feed intake and utilisation efficiency may be due to the dilution effect of fibrous feedstuff on diets. Indeed, fibre increases the bulkiness of a diet and, overtime, reduces the amount of feed consumed by birds, thereby imposing a physical limitation upon the intake of digestible nutrients (Guluwa *et al*., 2014). This is due to low absolute and relative GIT in growing birds, that limits the passage rate of the digesta.

Thus, this study represents the first attempt to investigate the feed value of APP in Jumbo quail diets. Repeated measures analysis revealed significant week and diet interaction effects on feed intake, weight gain and G:F, which indicates that the ability of the birds to utilize and convert the dietary treatments into body mass varied with their age. Indeed, at two weeks of age, feed intake linearly increased with APP levels, which indicates that the inclusion of the pomace does not compromise feed consumption in young quail. However, in week three, incremental levels of dietary APP resulted in a significant quadratic trend for feed intake. This indicates that an increase in feed consumption could be due to the increase in dietary fibre and polyphenols as APP levels increased. However, a gradual decrease in feed consumption was observed, suggesting that the young birds had a challenge in utilizing the diets as APP levels increased. This could be because young birds have lower absolute and relative gastrointestinal tract volume compared to older birds, hence the chicks were more affected by high dietary fibre (Murawska, 2017). Neither linear nor quadratic effects were recorded for feed intake in weeks 4 and 5.

Weight gain showed linear decreases in weeks 2, 3 and 4 of age, when APP was included up to 100 g/kg, indicating that the observed increase in feed intake was a nutrient dilution mechanism by birds to cope with the high levels of dietary fibre. Usually, when fibrous diets are fed to simple non-ruminants like poultry, the birds will respond by consuming more feed as a strategy to compensate for nutrient deficiency, however, over time fibre slows down the rate of passage of the digesta, but as soon as the gut is emptied the birds will continue to consume more feed (Mahlake *et al*., 2021). Indeed, high dietary fibre levels gradually decrease the relative amount of digestible dry matter in the diets, which causes compensatory feed intake (Murawska, 2017). These findings concur with the findings of Matoo *et al*. (2001), who reported a reduction in body weight gain in broiler chicks when dried AP was included at a rate of up to 50 g/kg in their diets. Similarly, Heidarisafar *et al*. (2016) reported reduced body weight in broilers fed with diets containing 100 g/kg of dried AP. At five weeks of age, weight gain tended to increase in the 75 g/kg APP group, which shows that the birds might have developed some coping mechanism to utilise high fibrous diets, which confirms the repeated measures outcome that the ability of the birds to utilize the diets depended on their age. This could have been facilitated by the development of the GIT. In similar studies, Biswas & Colombino *et al*. (2020) as well as Kilinc & Ayhan (2002), reported an improvement in weight gain in 5-weeks-old broiler chickens and quail birds reared on dried apple and tomato pomaces up to 100 g/kg and 150 g/kg inclusion level. The improved weight gains could be due to the difference in nutritional composition of the two pomaces. In addition, Joshi *et al*. (2010) investigated the effect of fermented AP on the performance of broiler chickens and indicated an increase in growth performance of birds reared on 100 g/kg AP, suggesting that pre-processing of the pomace could improve its utilisation. This requires further research to determine the effect of different processing strategies in valorising the feed value of APP for the Jumbo quail.

Linear decreases were observed for G:F in weeks 2, 3 and 4 as dietary APP levels increased, which corroborate the observed reduction in weight gain. This could be related to high dietary fibre content available in AP, which may gradually reduce feed utilisation efficiency in young quail (Mahlake *et al*., 2021). The decrease in G:F in weeks 2, 3 and 4 indicates the inability of the young birds to convert feed into muscle gain, which could have been due to poor utilisation of nutrients as a result of gut restriction. The decrease in G:F indicate poor utilisation of nutrients due to gut restriction. Similarly, Aghili *et al*. (2019) reported reduced FCE in two-week-old broilers fed with 120 g/kg of AP extract-containing feed. This could supports the decreases observed for G:F values in weeks 2 - 4 of this study. This further, corroborates the findings of Colombino *et al*. (2020), who reported that the supplementation of dried AP at 100 g/kg does not improve gain-to-feed ratio in broiler chickens. Nonetheless, a quadratic response for feed intake in week 5 could indicate the developed GIT for the maturing birds or could mean that the birds have developed a coping mechanism to utilise high dietary fibre.

3.4.2 Serum biochemical and haematological parameters

Blood analysis is a common tool for diagnosing clinical diseases as well as monitoring the impact of therapeutic, nutritional, and environmental management (Peres *et al*., 2015). Blood indices can also be used to determine the health status of animals in cases of poor nutrition, chronic pathology, or stress conditions that do not result in clinical symptoms. Haematological and serum biochemical analyses are routine practices for domestic and farm animals and provide significant diagnostic information about an animal's pathophysiological and health status (Casanovas *et al*., 2021). Blood analysis in poultry species is a quick and non-lethal tool for detecting malnutrition, stress and infections that could reduce productivity (Gutyj *et al*., 2019). In the current study, no significant linear or quadratic impacts were detected for any of the serum biochemical parameters, except for serum phosphorus and calcium, which linearly increased with dietary APP levels. In biochemical tests, the amount of calcium is important for the egg production and bone structure of quail birds while serum phosphorus is needed for the growth, maintenance, and repair of all tissues and cells (Sahin *et al*., 2007). In the current study, the linear increase in both blood calcium and phosphorus indicates that APP supplied more dietary calcium and phosphorus. Likewise, Ghazalah *et al*. (2011) reported an increase in blood phosphorus and calcium when dried AP was fed to broiler chickens at the rate of 20 g/kg. The authors elucidated that this could be influenced by the possible mechanism of antioxidant and antiperoxide activity of AP. All the parameters were within the normal physiological range for healthy quail birds (Mbhele *et al*., 2019; Fikry *et al*., 2021). This implies that the inclusion of APP had no negative effect on the health and nutritional status of the birds. Indeed, these results concur with those of Martín-Sánchez *et al.* (2014) and Bolacali *et al*. (2021), who reported a normal blood range in quail fed with dietary date palm (*Phoenix dactylifera*) extract at the rate of 7.5 g/kg.

Furthermore, dietary influences were observed on calcium, alkaline phosphatase (ALKP), choline and lipase. The quail reared on diet AP25 and AP50 had higher calcium content than the control diet, which could be attributed to the linear increase in feed intake in week 2, 3 and 4. The higher concentration of serum ALKP in the quail receiving the diets AP75 and AP100 may be linked to the liver damages caused by the consumption of APP polyphenols at higher levels AP75 and AP100, respectively. Increasing the dietary polyphenols content of fruit pomaces such as AP has been reported to lead to an increase in the production of short chain fatty acids, particularly propionate in the hindgut of monogastric animals (Millet *et al*., 2010). Therefore, since propionate plays an extremely important function as a precursor for liver functioning, the higher amount of propionate generated and absorbed in the ceca will enable the quail to synthesize more alkaline phosphatase in the liver, leading to an increased blood ALKP concentration. Ansar *et al*. (2004) reported a higher significant difference on serum phosphorus level in broiler chicks fed a control diet, which was due to the diets containing high levels of Ca. In the current study however, the reasons for an increase in phosphorus are unknown given that the control diet had a higher P level.

Haematological parameters are essential indicators of physiological, health, pathological and nutritional status of animals (Ewuola *et al*., 2004; Aro *et al*., 2013) because they provide distinct diagnosis and a quantifiable means of disease observation in animals. In this study, there were effects on platelet count, lymphocytes and heterophils in response to dietary incremental levels of APP. Platelets are defined as colourless blood cells that aid in blood clotting (Soslau, 2020). In this study, platelet count linearly declined which may have been caused by the impaired production as a result of the liver damages. Furthermore, a linear increase was observed for lymphocytes, which indicates the ability of the quail to produce antibodies for protection (Mahlake *et al*., 2021). Haematocrits measure the percentage of blood volume that consists of red blood cells after being centrifuged (Fradson, 1992). The haematocrit value is influenced by the number and size of red blood cells (Anggraeni *et al*., 2016). Haematocrit values in this study ranged from 43.53 to 47.07%, which is above the normal haematocrit value of 37% reported for quail (Sturkie & Griminger, 1976). However, the values were similar across the dietary treatments which indicate that the higher values were not caused by the inclusion of APP.

Heterophils showed negative quadratic trends as dietary APP level increased, indicating that the generation of the primary innate response to pathogens in the birds was activated (Achilonu *et al*., 2018). The highest APP inclusion levels (AP75 and AP100) displayed higher heterophils content compared to the control diet indicating the potential of APP biochemicals to boost the bird's immunity. This could be the reason why quadratic responses were observed for heterophils, which also concur with the findings of Achilonu *et al*. (2018), who reported that the inclusion of pumpkin (*Cucurbitaeae* sp.) seed meals at 100 g/kg increased and gradually decreased heterophils level in 6-weeks-old broiler chickens. Lymphocytes and heterophils are small white blood cells that plays a large role in defending the body against disease by attacking pathogenic bacteria and viruses that invade the body (Chuang *et al*., 2007). Moreover, Tanwar & Mishra (2001) reported that heterophils are actively amoeboid and phagocytic in nature, meaning that they could easily phagocyte other foreign cells that may be a threat to the bird.

3.5 Conclusion

Organic matter digestibility (OMD) increased with increasing APP levels. Moreover, feed intake increased but weight gain and G:F reduced for the young quail. However, an increase in G:F was observed for the maturing birds as shown by the quadratic response in week 5, from which an optimum inclusion level was calculated to be 50.0 g/kg. The inclusion level of APP at the rate greater than 50.0 g/kg reduced weight gain in weeks 2, 3 and 4, indicating that it compromised production performance of Jumbo quail. This potential reduction in bird performance at inclusion rates greater than 50.0 g/kg could be due to the presence of anti-nutritional effects such as saponins, tannins, glucosinolates, phytates and high fibre. Thus, the use of exogenous fibrolytic enzyme, fermentation and tannin-binding agents can be evaluated should higher levels beyond the optimum (50 g/kg) be desired.

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4 CHAPTER FOUR – CARCASS CHARACTERISTICS, INTERNAL ORGAN DEVELOPMENT, AND BREAST MEAT QUALITY AND COMPOSITION OF JUMBO QUAIL FED WITH APPLE POMACE-CONTAINING DIETS

Abstract

The utilization of apple pomace as a nutraceutical in Jumbo quail diets could promote sustainable production of safe and high-quality products while protecting the environment from wanton waste disposal in landfills. This study evaluated the effect of feeding graded levels of apple pomace powder (APP) on carcass characteristics, internal organs, breast meat quality and stability, and meat composition in Jumbo quail. Five isoproteic and isocaloric experimental diets were formulated by including APP in a standard grower diet at a rate of 0 (AP0), 25 (AP25), 50 (AP50), 75 (AP75), and 100 g/kg (AP100). Three hundred and fifty birds were equally allotted to the 5 experimental diets, producing 7 replicates as described in Chapter 3. At 35 days of age, 280 quail were slaughtered at a local poultry abattoir for the measurement of visceral organs, carcass traits, meat quality attributes, shelf life, and meat composition. There were linear decreases for hot carcass weight (HCW) [R^2 = 0.574; P = 0.0001], cold carcass weight (CCW) [R^2 = 0.577; P = 0.0001] and final body weight (FBW) [R^2 = 0.514; P = 0.0001] as dietary APP levels increased. There were neither linear nor quadratic trends observed for dressing percentage. Dietary APP levels linearly reduced drumstick weight [R^2 = 0.389; P = 0.0001], thigh [R^2 = 0.283; P = 0.002] and breast weights [R^2 = 0.432; P = 0.0001]. Small intestine length [R^2 = 0.229; P = 0.005] and weight [R^2 = 0.139; P = 0.046] linearly increased with increasing dietary APP levels. However, proventriculus showed a negative linear and quadratic effect [R^2 = 0.319; P = 0.017] with increasing dietary APP levels. Storage at room temperature affected shelf-life indicators (pH, temperature, lightness (L*), redness (a*) and yellowness (b*)). Increasing levels of APP resulted in a quadratic trend for breast meat crude protein (CP) [R^2 = 0.195; P = 0.017]and a linear decrease for meat ether extract (EE) [R^2 = 0.247; P = 0.004]. Quail fed with diet AP100 displayed higher breast meat crude fibre than those fed with diets AP25 and AP75. It can be concluded that using APP as a feed ingredient reduced carcass weights, carcass cuts and altered the sizes of some internal organs, and breast meat quality parameters.

Keywords: Bioactive substances, Carcass traits, Internal organs, Meat composition, Meat quality

4.1 Introduction

Quail rearing for meat and egg production is steadily becoming an economically viable business around the world (Redoy *et al*., 2017; Cavalcante *et al*., 2022). From the technical and economic viewpoints, quail rearing is attractive due to the birds' rapid growth rates, low feed requirements, high reproduction rates, and early onset of lay (Kar *et al*., 2017; Hamad & Kareem, 2019). Quail are birds of economic importance due to a series of positive features such as high meat quality and egg production, as well as fast returns on investment, which is made possible by their early sexual maturity, fast growth rates, short generation intervals, high laying rates, high feed efficiency, and limited space required per bird (Silva *et al*., 2018; Mnisi *et al*., 2021). Unfortunately, the over dependence on major conventional feed ingredients like maize and soybeans during feed formulation is unsustainable (Charlton *et al*., 2015). Further, maize and soybean cultivation require substantial amounts of water and hectares of land to grow, and have negative environmental impacts (Marareni & Mnisi, 2020). These negative environmental impacts include air pollution caused by land preparation activities, leaching of nutrients into water streams caused by the application of fertilizers and the distraction of the ozone layer caused by higher levels of atmospheric carbon due to land preparation activities. However, the reliance on these ingredients is because the commercial game-bird diet recommended for optimum quail production is expensive and not easily accessible (Mahlake *et al*., 2021; Mulaudzi *et al*., 2022).

Thus, the use of novel feed ingredients such as apple pomace (AP) in quail diets can promote the sustainability of quail production while protecting the environment. Indeed, the incorporation of AP in quail diets could address the augmented pressure on natural resources such as water and land, and also play a role in reducing feed-food competitions (Sekaran *et al*., 2021). Further, the incorporation of locally available feedstuffs such as AP could contribute to large-scale intensification of the Jumbo quail. The inclusion of AP has the potential to improve the quality of poultry products for human consumption (Granato *et al*., 2020). This is because AP is rich in protein, polyunsaturated fatty acids, polysaccharides, minerals, carotenoids and vitamins (Helkar *et al*., 2016; Tlais *et al*., 2020), and contain bioactive compounds with antioxidant, anti-inflammatory, antiproliferative and antimicrobial activities (Kowalska *et al*., 2017). Compounds such as antioxidants are commonly used to prevent oxidation and preserve sensory properties (Walia *et al*., 2019) and, as such, improve meat quality. However, synthetic antioxidants such butylated hydroxytoluene, butylated hydroxyanisol, as well as nitrites and nitrates, have all been implicated in the development of stomach, bowel, and food allergies in humans (Pereira & Maraschin., 2015; Naseri *et al*., 2018).

In this context, the use of natural antioxidants in meat products could be a viable strategy to reduce the use of synthetic additives because natural additives exhibit low toxicity, in addition to carrying out functional activities that are beneficial to human health (Carocho *et al*., 2014; Ribeiro *et al*., 2019). Therefore, the use of AP in Jumbo quail diets could be a long-term strategy to produce meat products with higher levels of bioactive compounds that have health benefits for consumers. To this end, there is scanty information on the influence of apple pomace powder (APP) inclusion in Jumbo quail diets on organ development, carcass characteristics, meat quality and stability, and meat composition. This study was, therefore, undertaken to evaluate the impact of graded levels of dietary APP on meat quality parameters, carcass traits, shelf-life indicators, meat composition, and internal organ sizes in Jumbo quail. It was hypothesized that the inclusion of dietary APP as a nutraceutical would improve internal organs, carcass traits, meat quality and stability, and meat composition in Jumbo quail.

4.2 Material and methods

4.2.1 Study site, diet formulation and experimental design

The present study was carried out at the North-West University experimental farm, Molelwane. The location and climatic conditions of the study site were as described in Chapter 3, Section 3.2.1. Diet formulation and experimental design were also as described in Chapter 3, Sections 3.2.5 and 3.2.6.

4.2.2 Feeding trial and slaughter protocol

The experimental design, commencement, and termination (i.e., slaughter procedures) of the feeding trial were as described in Chapter 3, Sections 3.2.7 and 3.2.8.

4.2.3 Carcass performance and internal organs

After slaughter, the carcasses were labelled per experimental unit and placed in tagged plastic bags. The carcasses were immediately weighed to determine hot carcass weight (HCW). After weighing, they were then taken to a cold room (16°C) for 24 h and then reweighed to determine cold carcass weight (CCW). Carcass cuts (wing, drumstick, breast, and thigh) and internal organs (liver, gizzard, proventriculus, spleen, small intestine, caecum, and colon) were weighed using a digital weighing scale (Explorer®EX224, OHAUS Corporation, Parsippany, NJ, USA) and expressed as a proportion of HCW (%HCW). Dressing percentage was calculated as the proportion of HCW on slaughter weight (final body weight) using the following formula:

$$Dressing\ percentage = \frac{hot\ carcass\ weight}{final\ body\ weight} \times 100$$

4.2.4 Meat pH and colour

A total of 280 breast meat samples (8 per pen) were used to analyse meat pH and colour. Breast meat pH was taken 1 h and 24 h after slaughter using a portable pH meter (HI98163, Hanna Instruments, Woonsocket, RI, USA) equipped with an electrode (Crison, Barcelona, Spain) that was inserted about 1.5 – 2.5 cm into the meat. Before measurements, a calibration of the instrument was performed by using standard phosphate buffers (pH 4.00, 7.00 and 10.00). After measuring each experimental unit, the electrode was carefully rinsed in distilled water and recalibrated. Colour coordinates (L^* = lightness, b^* = yellowness and a^* = redness) were measured on the surface of a freshly cut slice of the breast muscle using a Konica Minolta Spectrophotometer (INC colour-guide, BYKGardener GmbH, Geretsried, Germany), with a 20 mm diameter measurement area, illuminant D65-day light, and 10° observation angle. The colour meter was calibrated before measurements and after measuring every experimental unit using the zero and white calibration as recommended by the manufacturer. Hue angle was calculated as $\tan^{-1}(b^*/a^*)$, and chroma values were calculated as $\sqrt{a*^2 + b*^2}$ as described by Priolo *et al*. (2002).

4.2.5 Water holding capacity and drip loss

A total of 70 breast meat samples (two per pen) were used to analyse water holding capacity and drip loss. Water holding capacity (WHC) was determined according to the procedure of Grau & Hamm (1953), using the pectoral major muscle (8 - 16 g), which was placed in-between two filter papers and held under pressure (60 kg of pressure) using dumbbell weights for 5 min. Thereafter, the meat sample was re-weighed. WHC was calculated using the equation:

$$Water\ holding\ capacity\ (\%) = [100 - (\frac{initial\ weight - weight\ after\ pressure}{initial\ weight} \times 100)]$$

Drip loss was performed using a method adapted from Zhang & Pan (2009). Breast meat samples were sliced with a knife into pieces weighing between 2 and 3 grams. The sample weights were recorded as initial weight. The sample was then hooked and suspended using wire steel in a plastic and sealed properly so that it does not touch the sides of the bottle. The bottles were then stored in cold room set at 4°C for 48 hours, after which the samples were taken out gently and wiped to remove any liquid on the surface and reweighed to obtain final weight. The difference in weight of each sample before and after drip in proportion to the initial weight was conveyed as percentage drip loss using the formula below:

$$Drip\ loss\ (\%) = \frac{initial\ weight\ of\ sample - final\ weight\ of\ sample}{initial\ weight\ of\ sample} \times 100$$

4.2.6 Shear force

Raw breast meat samples were used to determine breast meat tenderness. The samples were sheared (crosshead speed 100 mm / min, one shear in the centre of each core) perpendicular to the direction of muscle fibres using a Meullenet-Owens Razor Shear Blade (A/MORS) mounted on a texture analyser (TA XT plus, Stable Micro Systems, Surrey, UK). The reported value represented the average peak shear force for each sample, which was expressed in Newtons.

4.2.7 Cooking loss

Raw breast muscle samples were chilled overnight at 4°C and were individually weighed to obtain initial weight before cooking. The samples were then placed in a foil plate and oven-cooked as described by Honikel (1998) to reach an internal temperature of 75°C. The cooked samples were then removed from the oven and left to cool for 20 min. The samples were then re-weighed to obtain the weight after cooking. The cooking loss was then calculated using the following equation:

$$\text{Cooking loss (\%)} = \frac{weight\ before\ cooking - weight\ after\ cooking}{weight\ before\ cooking} \times 100$$

4.2.8 Meat chemical composition

Breast meat samples were used to analyse chemical composition of the quail breast. The proximate composition of the breast meat samples was analysed in accordance with AOAC (2005). Raw meat samples were deboned, dried and ground (Quaresma *et al*., 2022) to produce powder. The breast powder was then analysed at the North-West University research farm Animal Nutrition laboratory for dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), ether extract (EE) and ash. The detailed procedure was described in Chapter 3, Section 3.2.4.

4.2.9 Meat shelf life

Randomly selected breast meat samples per experimental unit were used for the determination of quail breast meat shelf life at room temperature (20 - 27°C). The breast meat samples were placed in a labelled foil plate and stored in a cool and dry area in the Meat Science laboratory of the North-West University. Meat pH and colour (lightness, redness, and yellowness) were then recorded daily for a period of 7 days. Hue angle and chroma values were calculated as described by Priolo *et al*. (2002).

4.3 Statistical analyses

Data on internal organs, carcass traits, meat quality and composition were evaluated for linear and quadratic effects using polynomial contrast in a response surface regression analysis (PROC RSREG; SAS, 2010) as described in Chapter 3, Section 3.2.10. Repeated measures analysis in PROC GLM was conducted for shelf-life data to evaluate the interaction effect between storage time × diet on pH, temperature, L^*, a^* and b^*. One-way ANOVA (PROC GLM; SAS, 2010) model employed in Chapter 3 was also used to determine the effects of APP inclusion on internal organs, carcass characteristics, and meat quality parameters. Significance was declared at $P < 0.05$, and least squares means were compared using the probability of difference option in SAS.

4.4 Results

4.4.1 Carcass characteristics

Table 4.1 shows the effect of APP-containing diets on carcass characteristics of Jumbo quail birds. There were significant linear decreases for HCW [y = 120.4 (±2.406) – 0.162 (±0.114) x; R^2 = 0.574; P = 0.0001], CCW [y = 101.01 (±2.394) – 0.123 (±0.114) x; R^2 = 0.577; P = 0.0001] and FBW [y = 183.4 (±3.184) - 0.041 (±0.151) x; R^2 = 0.514; P = 0.0001] as dietary APP levels increased. There were neither linear nor quadratic trends observed for dressing percentage in response to increasing levels of dietary APP. There were no significant linear or quadratic effects observed for all the carcass cuts except for drumstick, thigh, and breast weights. Dietary APP levels reduced drumstick weight [y = 2.941 (±0.074) – 0.004 (±0.0035) x; R^2 = 0.389; P = 0.0001], thigh weight [y = 4.27 (±0.167) – 0.002 (±0.008) x; R^2 = 0.283; P = 0.002] and breast weight [y = 11.8 (±0.399) – 0.026 (±0.0189) x; R^2 = 0.432; P = 0.0001] as APP levels increased.

Jumbo quail fed diet AP0 produced heavier HCW (122.2 g) while those fed AP100 promoted the lowest HCW (97.36 g). However, birds fed with diets AP25, AP50 and AP75 had similar (P >0.05) HCW. Likewise, diet AP0 promoted higher CCW (100.9 g) than diets AP75 (88.34 g) and AP100 (79.45 g), which did not differ (P >0.05). Nonetheless, the control diet AP0 promoted similar CCW as diets AP25 and AP50, whose CCW values were statistically similar (P >0.05) to diet AP75. Quail fed diet AP0 had heavier drumstick weight (2.97 %HCW) compared to quail fed diets AP50 and AP100, whose drumstick weights did not differ (P >0.05). Nonetheless, diet AP0 promoted similar (P >0.05) drumstick weight as diets AP25 and AP75. Quail fed diet AP75 also showed similar (P >0.05) drumstick length as quail on diets AP50 and AP100. Diet AP100 promoted the lowest thigh weight (3.39 %HCW) when compared with all other dietary treatments, which produced similar (P >0.05) thigh weight. Diet AP0 produced the heaviest breast weights (11.90 %HCW) while diet AP100 produced the lowest breast weights (09.01 %HCW). Diets AP0, AP25 and AP50 promoted similar (*P* >0.05) breast weights. Diet AP75 also promoted the same (*P* >0.05) breast weight as diets AP25 and AP50. Birds reared on diet AP0 had the highest final body weight (FBW) (185.9 g/bird) than those on diets AP50 and AP100, whose FBW was different. Nonetheless, the control diet AP0 promoted similar (*P* >0.05) FBW as diets AP25 and AP75.

Table 4.1. Carcass characteristics (%HCW, unless otherwise stated) of Jumbo quail reared on diets containing apple pomace powder.

	[1]Diets						Significance		
[2]Parameters	AP0	AP25	AP50	AP75	AP100	[3]SEM	*P* value	Linear	Quadratic
Dressing (%)	65.74	62.77	63.80	60.44	62.73	1.616	0.252	0.111	0.318
HCW (g)	122.2^{c}	112.4^{b}	111.0^{b}	109.0^{b}	97.36^{a}	2.427	0.0001	0.0001	0.574
CCW (g)	100.9^{c}	98.16^{bc}	90.95^{bc}	88.34^{ab}	79.45^{a}	2.589	0.0001	0.0001	0.426
FBW (g)	185.9^{c}	179.2^{bc}	174.1^{b}	180.3^{bc}	155.9^{a}	2.731	0.0001	0.0001	0.065
Drumstick (cm)	5.559	5.419	5.381	5.410	5.402	0.072	0.441	0.157	0.221
Drumstick	2.965^{b}	2.810^{b}	2.654^{a}	2.729^{ab}	2.444^{a}	0.077	0.001	0.0001	0.924
Thigh (cm)	4.571	4.379	4.776	4.649	4.469	0.186	0.607	0.913	0.482
Thigh	4.405^{b}	3.887^{b}	4.142^{b}	3.976^{b}	3.393^{a}	0.168	0.003	0.002	0.413
Wing (cm)	10.99	10.72	10.85	10.87	10.35	0.207	0.237	0.103	0.091
Wing	3.388	3.461	3.242	3.596	3.002	0.215	0.369	0.361	0.355
Back (cm)	9.551	9.247	9.156	9.328	9.357	0.451	0.979	0.826	0.575
Breast	11.90^{c}	10.93^{bc}	10.43^{abc}	10.10^{ab}	09.01^{a}	0.431	0.001	0.0001	0.967

^{a,b,c} In a row, means with common superscripts do not differ (*P* >0.05).
[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]Parameters: HCW = hot carcass weight, CCW = cold carcass weight, FBW = final body weight.
[3]SEM: standard error of the mean.

Table 4.2 shows the effect of APP-containing diets on visceral organ sizes of Jumbo quail. There were no significant linear or quadratic effects observed for all internal organ sizes except for the length and weight of small intestine and proventriculus weights in response to increasing APP levels. Small intestine length [$y = 0.029\ (\pm0.064)\ x + 68.4\ (\pm1.35)$; $R^2 = 0.229$; $P = 0.005$] and weight [$y = 0.012\ (\pm0.008)\ x + 5.72\ (\pm0.177)$; $R^2 = 0.139$; $P = 0.046$] linearly increased with increasing dietary APP levels. However, proventriculus showed a negative linear and quadratic effect [$y = 0.00003\ (\pm0.00001)\ x^2 – 0.004\ (\pm0.001)\ x + 0.75\ (\pm0.025)$; $R^2 = 0.319$; $P = 0.017$] with increasing dietary APP levels. Dietary influences were only observed on the proventriculus, where birds reared on diet AP0 promoted the heaviest proventriculus weight (0.778 %HCW) compared to all other diets, which did not differ (P >0.05).

Table 4.2. Effects of feeding graded levels of apple pomace powder on visceral organ sizes (%HCW, unless stated otherwise) in Jumbo quail.

[2]Parameters	[1]Diets					[3]SEM	Significance		
	AP0	AP25	AP50	AP75	AP100		*P* value	Linear	Quadratic
Proventriculus	0.778[b]	0.608[a]	0.632[a]	0.677[a]	0.621[a]	0.021	0.0001	0.006	0.017
Gizzard	3.234	2.853	2.973	3.098	3.050	0.091	0.070	0.689	0.072
Liver	3.030	3.063	2.995	3.032	2.970	0.107	0.977	0.648	0.831
Spleen	0.188	0.208	0.201	0.249	0.196	0.017	0.119	0.316	0.172
SI (cm)	68.01	69.97	70.65	71.12	74.36	1.464	0.065	0.005	0.668
SI	5.684	6.108	6.005	6.300	6.204	0.189	0.211	0.046	0.366
Caecum (cm)	10.27	10.10	9.90	10.24	10.20	0.307	0.9115	0.989	0.479
Caecum	1.082	1.097	1.059	1.586	1.437	1.152	0.112	0.209	0.680

[a,b,c] In a row, means with common superscripts do not differ ($P > 0.05$).

[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.

[2]Parameters: SI = small intestine.

[3]SEM: standard error of the mean.

4.4.2 Meat quality parameters

Table 4.3 indicates that there were no linear nor quadratic effects (*P* >0.05) for all breast meat quality parameters, except for 1-hour yellowness (b^*_1) and 24-hour lightness (L^*_{24}), redness (a^*_{24}) and $chroma_{24}$. Likewise, no linear or quadratic effects (*P* >0.05) were observed for shear force. Linear increases were recorded for b^*_1 [$y = 0.001 (\pm 0.015) x + 2.97 (\pm 0.312)$; $R^2 = 0.156$; $P = 0.028$] and L^*_{24} [$y = 0.009 (\pm 0.051) x + 41.7 (\pm 1.078)$; $R^2 = 0.130$; $P = 0.050$] as a result of increasing dietary APP levels. However, a^*_{24} [$y = 5.69 (\pm 0.179) – 0.018 (\pm 0.009) x$; $R^2 = 0.251$; $P = 0.005$] and $chroma_{24}$ [$y = 6.71 (\pm 0.209) – 0.017 (\pm 0.010) x$; $R^2 = 0.282$; $P = 0.002$] linearly declined with increasing APP levels.

Table 4.3 shows that there were significant dietary effects for pH_{24}, L^*_1, a^*_{24} and $chroma_{24}$. Diet AP25 promoted higher pH_{24} value (5.587) when compared to diets AP0, AP50 and AP100, which did not differ (*P* >0.05). Diet AP75 promoted similar pH_{24} values as all the other treatment groups. Breast meat from birds reared on diet AP25 had higher L^*_{24} value (44.0) than breast meat from those reared on diets AP50 and AP100, which did not differ (*P* >0.05). Breast meat L^*_{24} of Jumbo quail fed with the control diet AP0 had similar (*P* >0.05) L^*_{24} with those fed with the other dietary treatments. Diet AP0 produced higher a^*_{24} value (5.461) than diets AP50 and AP100, which produced similar a^*_{24} (*P* >0.05). Moreover, the a^*_{24} was similar for quail meat from the AP0, AP25 and AP75 groups. Moreover, diet AP75 showed similar a^*_{24} as diets AP50 and AP100. At 24-hour post-mortem, diets AP0 and AP25 promoted higher chroma values than diets AP50 and AP100. Diet AP75 promoted similar (*P* >0.05) $chroma_{24}$ values as, the other dietary treatment groups.

Table 4.3. The effect of apple pomace-containing diets on meat quality parameters in Jumbo quail birds.

[2]Parameters	[1]Diets					[3]SEM	Significance		
	AP0	AP25	AP50	AP75	AP100		*P* value	Linear	Quadratic
pH_1	5.954	5.959	5.836	5.996	5.949	0.053	0.315	0.908	0.376
pH_{24}	5.119^{a}	5.587^{b}	5.01^{a}	5.241^{ab}	5.053^{a}	0.102	0.003	0.227	0.279
$Temp_1$	22.04	20.49	18.14	22.51	20.90	1.178	0.106	0.943	0.169
$Temp_{24}$	19.17	20.58	19.14	20.55	18.95	0.871	0.490	0.867	0.339
L^*_1	48.99	48.79	48.99	48.36	48.81	1.052	0.993	0.805	0.900
L^*_{24}	40.86^{ab}	44.00^{b}	39.68^{a}	40.05^{ab}	39.14^{a}	1.056	0.023	0.050	0.434
a^*_1	6.077	5.454	5.402	5.161	6.238	0.584	0.641	0.987	0.141
a^*_{24}	5.641^{c}	5.52^{bc}	4.76^{a}	5.16^{abc}	4.91^{ab}	0.179	0.006	0.005	0.209
b^*_1	2.909	3.007	2.938	2.147	2.129	0.335	0.158	0.028	0.448
b^*_{24}	3.397	3.557	2.997	2.935	2.826	0.357	0.544	0.121	0.975
$Chroma_1$	6.759	6.301	6.216	5.667	6.712	0.550	0.634	0.674	0.218
$Chroma_{24}$	6.62^{b}	6.63^{b}	5.70^{a}	6.01^{ab}	5.71^{a}	0.211	0.004	0.002	0.458
Hue $angle_1$	0.443	0.495	0.499	0.396	0.386	0.059	0.523	0.254	0.294
Hue $angle_{24}$	0.537	0.574	0.553	0.508	0.516	0.056	0.923	0.538	0.696
WHC (%)	90.76	87.70	91.57	86.31	89.01	1.367	0.064	0.309	0.675
Drip loss (%)	4.697	4.857	3.898	3.908	4.556	0.442	0.401	0.385	0.248
CKL (%)	39.67	43.71	39.25	41.35	42.90	2.998	0.791	0.666	0.888
Shear force (N)	3.022	2.944	3.364	3.274	3.303	0.274	0.763	0.301	0.771

[a,b,c] In a row, means with common superscripts do not differ (P >0.05).
[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]Parameters: WHC = water holding capacity, L^* = lightness, a^* = redness, b^* = yellowness, CKL = cooking loss.
[3]SEM: standard error of the mean.

4.4.3 Breast meat shelf-life measurement

Repeated measures analysis for shelf life showed no week × diet interaction effects on pH (*P* = 0.166), temperature (*P* = 0.427) and *a** (*P* = 0.071). However, a week × diet interaction effects were observed on *L** (*P* = 0.0003) and *b** (*P* = 0.015). Figure 4.1 shows the effect of diets containing incremental levels of APP on breast meat pH in Jumbo quail. There were no linear or quadratic trends (*P* >0.05) on days 1, 2, 3, 4, 5 and 6 for pH in response to increasing APP levels. However, on day 7, there was a linear increase [$y = 0.007\ (\pm 0.013)\ x + 7.78\ (\pm 0.303)$; $R^2 = 0.239$; *P* = 0.039] as a result of increasing dietary APP levels.

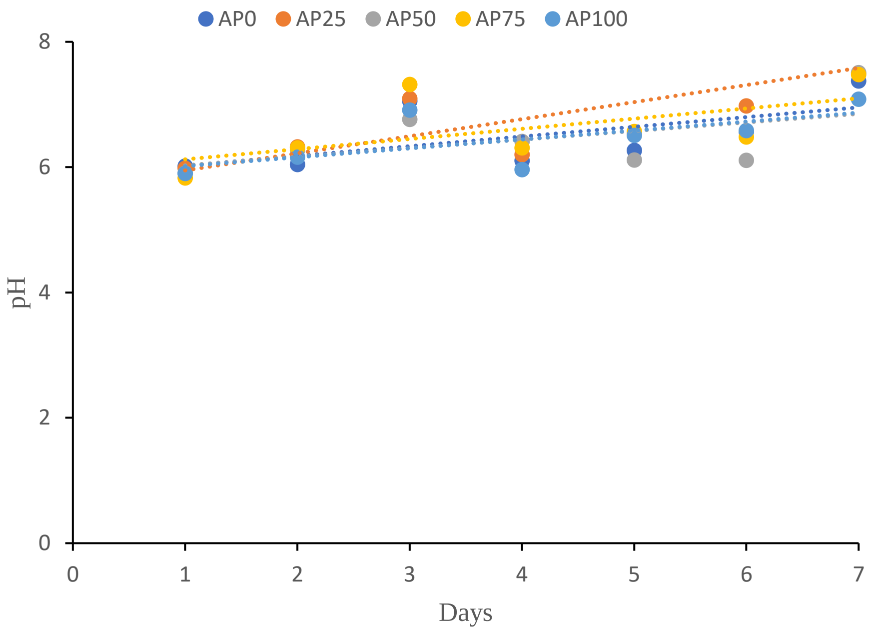

Figure 4.1. The effect of apple pomace-containing diets on the stability of breast meat pH upon storage at room temperature for 7 days [AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder].

Figure 4.2 shows the effect of diets containing incremental levels of APP on the breast meat temperature in Jumbo quail. There were no linear or quadratic trends (*P* >0.05) on days 1, 4, 5, 6 and 7 for temperature in response to increasing APP levels. There were linear [$R^2 = 0.631$; *P*

= 0.0001] and quadratic effects [R^2 = 0.631; P = 0.018] for temperature on day 2, while on day 3, a linear decline was observed on temperature [R^2 = 0.338; P = 0.005] APP levels increased. Breast meat sample of quail reared on diet AP0 promoted higher temperature (23.19 °C) on day 2 of storage than those reared on diets AP50, AP75 and AP100, which had similar (P >0.05) temperature on day 2. On day 3, the highest temperature was from breast meat sample of quail on diet AP100 (24.46 °C) when compared to diets AP0 and AP25, which displayed a similar (P >0.05) temperature.

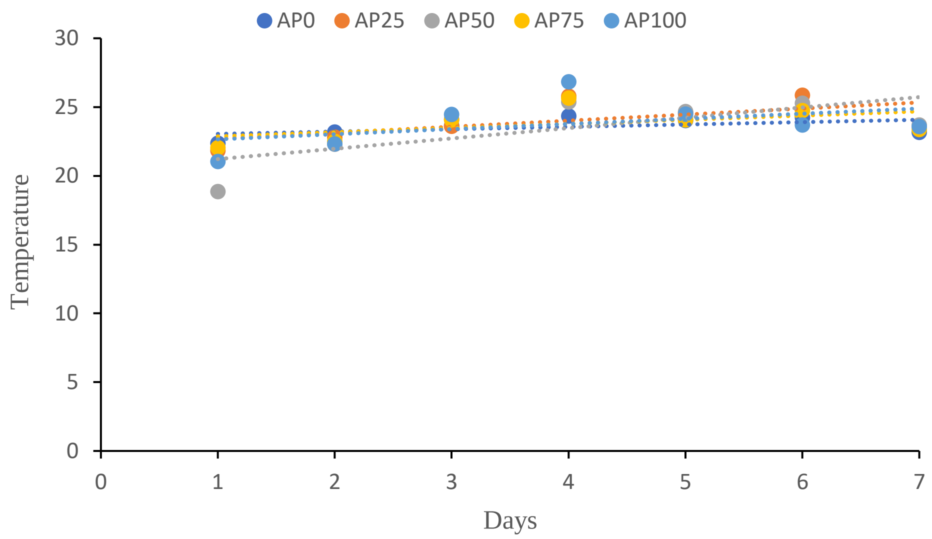

Figure 4.2. The effect of apple pomace-containing diets on the stability of breast meat temperature upon storage at room temperature for 7 days [AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder].

Figure 4.3 shows the effect of diets containing incremental levels of APP on breast meat lightness (L*) in Jumbo quail. There were no linear or quadratic trends (P >0.05) on days 1, 2, 5 and day 7 for breast meat L* in response to increasing dietary APP levels. On day 3, significant quadratic effects were observed on L* [R^2 = 0.237; P = 0.044]. On day 4, positive linear [R^2 = 0.437; P = 0.005] and quadratic trends [R^2 = 0.437; P = 0.029] were observed for L^*, while, on day 6, negative linear [R^2 = 0.354; P = 0.019] and quadratic effects [R^2 = 0.354; P = 0.046] were recorded. The L^* of the breast meat colour on day 7 was lowest for quail birds fed with diet AP100 (18.69) when compared with all the other breast samples from the other diets, which did not differ (P >0.05).

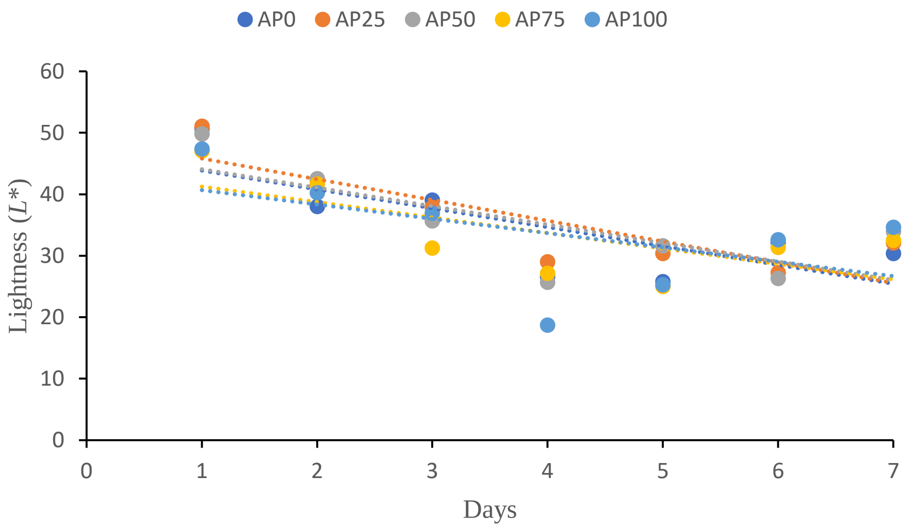

Figure 4.3. The effect of apple pomace-containing diets on the stability of breast meat lightness upon storage at room temperature for 7 days [AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder].

Figure 4.4 shows the effect of diets containing incremental levels of APP on the breast meat redness (a^*) of Jumbo quail birds. There were no linear or quadratic trends (P >0.05) on days 1, 2, 3, 5, 6 and 7 for a^* in response to increasing APP levels. Negative linear [R^2 = 0.63; P = 0.008] and quadratic effects [R^2 = 0.633; P = 0.0001] were observed for a^* on day 4 in response to dietary APP levels. On day 5, the a^* was higher for breast sample from the AP50 group (11.48) than breast sample from the AP0 and AP25 groups.

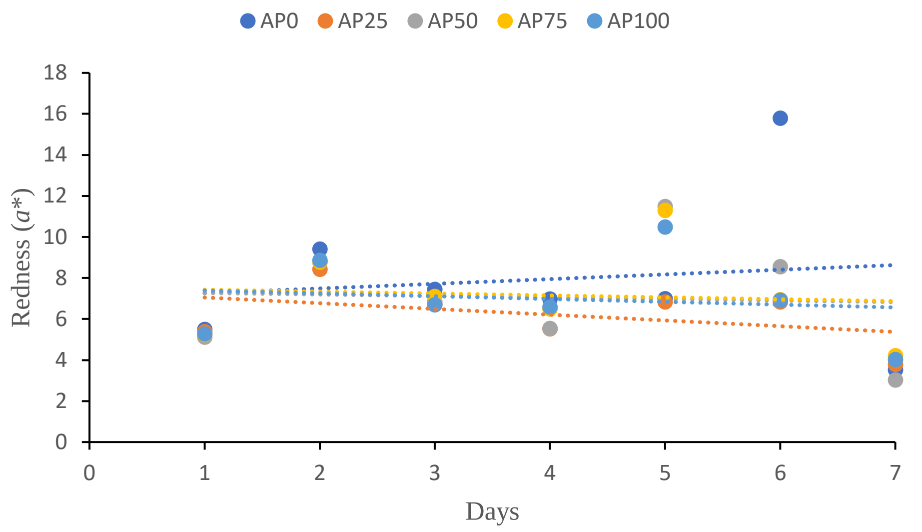

Figure 4.4. The effect of apple pomace-containing diets on the stability of breast meat redness upon storage at room temperature for 7 days [AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder].

Figure 4.5 shows the effect of diets containing incremental levels of APP on breast meat yellowness (*b**) in Jumbo quail. There were no linear or quadratic effects (P >0.05) on days 4, 5, 6 and 7 for *b** in response to increasing dietary APP levels. On day 1, *b** linearly declined [R^2 = 0.236; P = 0.038], while, on day 2, a positive quadratic response [R^2 = 0.268; P = 0.017] was recorded with increasing dietary APP levels. On day 3, *b** linearly increased [R^2 = 0.526; P = 0.0001] with increasing dietary APP levels. Diet AP100 promoted higher *b** value (5.49) than diet AP25 (3.61) in day 3, while in day 4, AP75 group showed higher *b** values (5.261) than the AP100 group (1.75). Likewise, diet AP50 (5.26) produced higher (P <0.05) *b** value than diet AP25 (2.53) in day 5.

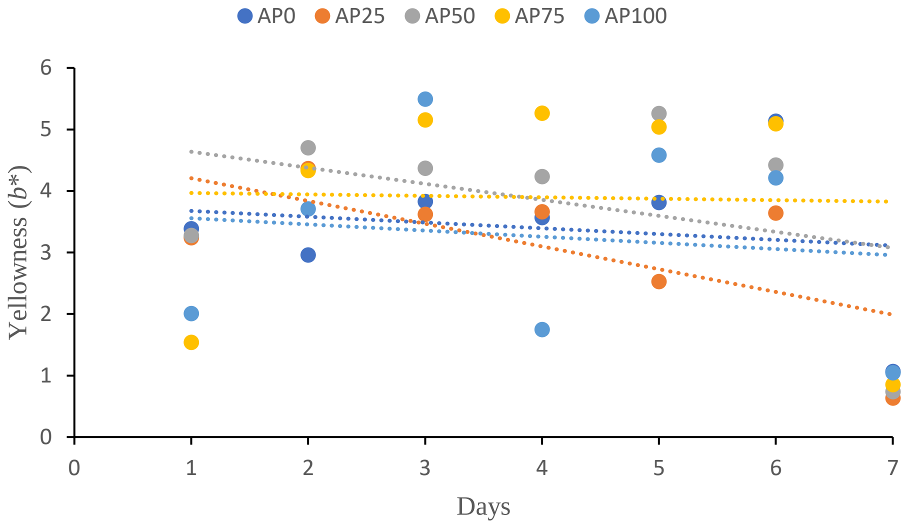

Figure 4.5. The effect of apple pomace-containing diets on the stability of breast meat yellowness upon storage at room temperature for 7 days [AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder, and AP100 = a standard grower diet with 100 g/kg of apple pomace powder].

4.4.4 Meat composition

Table 4.4 shows that there neither linear nor quadratic effects observed for meat dry matter (DM), organic matter (OM), crude fibre (CF) and ash contents in response to incremental levels of APP. However, increasing levels of APP resulted in a positive quadratic effect for crude protein (CP) [$y = 208.1\ (\pm 7.44) + 0.733\ (\pm 0.353)\ x - 0.01\ (\pm 0.003)\ x^2$; $R^2 = 0.195$; $P = 0.017$] and a linear decrease for meat ether extract (EE) [$y = 36.1\ (\pm 3.608) - 0.002\ (\pm 0.171)\ x$; $R^2 = 0.247$; $P = 0.004$]. Quail birds fed diet AP75 had higher (P <0.05) meat CP (225.9 g/kg) than those fed with diets AP0, AP50 and AP100, which did not differ (P >0.05). However, birds fed diets AP25 had similar (P <0.05) CP content with those fed diet AP75. Breast meat from quail on diet AP100 had higher (P <0.05) meat EE value (49.44 g/kg) than meat from those on diet AP25. However, quail on diet AP100 promoted similar (P >0.05) meat EE content with those reared on diets AP0, AP50 and AP75.

Table 4.4. Effect of apple pomace-containing diets on breast meat composition (g/kg DM, unless stated otherwise) in Jumbo quail.

[2]Components	[1]Diets					[3]SEM	Significance		
	AP0	AP25	AP50	AP75	AP100		*P* value	Linear	Quadratic
DM (g/kg)	274.5	222.3	250.8	246.0	257.9	14.77	0.186	0.844	0.103
OM	238.1	181.1	208.3	217.4	224.3	17.63	0.240	0.881	0.112
CP	207.8^{ab}	225.4^{b}	212.1^{ab}	225.9^{b}	192.7^{a}	7.580	0.023	0.245	0.017
CF	18.04	17.62	15.93	17.43	18.99	1.028	0.345	0.597	0.068
EE	38.00^{ab}	33.08^{a}	40.33^{ab}	47.79^{ab}	49.44^{b}	3.818	0.026	0.004	0.359
Ash	36.36	41.19	42.47	28.57	33.63	10.03	0.862	0.566	0.692

[1]Diets: AP0 = a standard grower diet with no apple pomace powder inclusion, AP25 = a standard grower diet with 25 g/kg of apple pomace powder, AP50 = a standard grower diet with 50 g/kg of apple pomace powder, AP75 = a standard grower diet with 75 g/kg of apple pomace powder and AP100 = a standard grower diet with 100 g/kg of apple pomace powder.
[2]Components: DM = dry matter, OM = organic matter, CP = crude protein, CF = crude fibre, EE = ether extract.
[3]SEM = standard error of the mean.

4.5 Discussion

4.5.1 Internal organs and carcass characteristics

The size of an animal's internal organs indicates its digestive health and function (Kuzmuk *et al*., 2005). Feed and housing systems all have an impact on the function and size of internal organs (Young *et al*., 2001; Havenstein *et al*., 2003; Brickett *et al*., 2007). A well-balanced diet and a conducive environment promote good health and productivity. Moreover, genetics, sex, slaughtering conditions, and age of the animal are some of the factors that influence organ development (Young *et al*., 2001). However, in this study, sizes of internal organs were not affected by diets, except for proventriculus and small intestine (weight and length). Small intestine length and weight linearly increased with increasing dietary APP levels. The enlargement of the small intestine could have been an anatomical and physiological mechanism to utilise high dietary fibre in the APP groups (Oliveira *et al*., 2008; Laudadio *et al*., 2012). Proventriculus linearly decreased with increasing APP levels, which could be attributed to the inability for the birds to digest high fibrous diets, which led to reduced feed intake, which was observed in young quail and, as a result, reduced body weight in the early stage of growth. The proventriculus is the first part of a bird's stomach, where digestive enzymes (hydrochloric acid and pepsinogen) are secreted and mixed with food before it goes to the gizzard (Mohamed, 2021)

Carcass yield and carcass weights are essential meat market characteristics because they are used to grade meat products and have a direct impact on market prices (Matshogo *et al*., 2020). The inclusion of APP decreased hot and cold carcass weights and also the final body weight. Cold carcass yield is believed to be an excellent predictor of total edible meat during storage. Therefore, this implies that adding APP may reduce the amount of edible meat from quail. These results agree with those of Habibian *et al.* (2016) and Zeferino *et al.* (2016), who reported that nutritional complications such as an ingredient source, shortage and incorrect dilution of nutrients may have a negative effect on carcass quality of quail. The decrease in carcass weights in this study could be due to the linear decreases in weight gain and G:F suggesting that the birds could not efficiently convert the APP-containing diets into body mass or for muscle development.

4.5.2 Meat quality

The assessment of meat quality parameters is performed to evaluate physical, biological and chemical characteristics of meat such as pH, tenderness, texture, colour and storage qualities (Elmasry *et al*., 2012; Melo *et al*., 2016). The addition of certain feed ingredients to poultry diets has a direct influence on meat quality, such as the physical characteristics and the chemical and nutritional composition of the meat, which affect its acceptance by consumers (Mazizi *et al*., 2019). In this study, neither the linear or quadratic effects were observed for all parameters except for 24-hour pH, lightness, redness and chroma. The quality of meat with regard to storage and processing depends on pH values and the water holding capacity (WHC). Dietary APP inclusion had an effect on breast meat pH 24-hour post-mortem with the highest pH observed on diet AP25 when compared to diet AP50. This could mean that APP had a positive effect on meat colour as influenced by the dietary polyphenols or anticyanins, which is associated with the darker colour observed on the current study.

Meat pH is important from meat quality perspective because it does not only affect the taste but has direct influence on the other meat quality traits such as WHC, drip loss, cooking loss, shelf life, tenderness, colour and juiciness (Muchenje *et al*., 2009a). For instance, several authors reported a significant correlation between post-mortem breast meat pH values and colour values, where a lower pH value results in lighter or pale meat, while a higher pH value causes dark meat colour (Barbut, 1993; Muchenje *et al*., 2009b). Indeed, these findings corroborates the ones observed from the current study where a higher pH value was observed at 24-h post-mortem, while a lower pH value was recorded at 1 h post-mortem. Lightness is a good indicator of the freshness of meat and has a direct influence on the final purchase decision of consumers (Mancini & Hunt, 2005). According to Qiao *et al*. (2002), meat lightness can be classified as pale ($L^* > 53$), normal ($48 < L^* < 51$) and dark ($L^* < 46$). Thus, the inclusion of APP promoted darker meat colour at 24 h post-mortem but tended to exhibit a normal colour at 1 h post-mortem. This represents the storage effect of the quail breast meat during a 7-day storage period. Generally, the increase in meat pH is associated with improved meat quality and also suggest microbial proliferation. These results agree with those of Genchev *et al*. (2008), who reported that meat from quail breasts is dark, with values of L^* less than 50.0, a^* greater than 4.5 and b^* less than 10.0. Dietary treatments had a significant effect on the red colour intensity of the breast meat, which could be attributed to the presence of anthocyanins found in AP, which cause meat pigmentation (Erinle & Adewole, 2022). These findings are in concordance with the results from Cavalcante *et al*. (2022), who reported an increase in red colour intensity of meat when Japanese quail were supplemented with sources of PUFAs from soybean oil, linseed oil and Brazilian nut oil. Shear force, a measure of meat tenderness, was not affected by the dietary treatments, suggesting that supplementing the diets with APP does not affect meat texture (Warner, 2017).

4.5.3 Meat shelf-life and composition

Repeated measures analysis for shelf life showed no week × diet interaction effects on pH (P = 0.166), temperature (P = 0.427) and a^* (P = 0.071). However, a week × diet interaction effects were observed on L^* (P = 0.0003) and b^* (P = 0.015). Apple pomace contains a variety of bioactive chemicals and natural preservatives that may extend the keeping quality of poultry products at room temperature (Sweeney & O'Doherty, 2016). The outcomes of this study indicated that adding APP to the diet had a significant effect on all shelf-life indicators during a 7-day storage period. A linear increase was observed for meat pH on day 7. This elevation in meat pH on day 7 is generally associated with improved microbial spoilage. Moreover, a microbial proliferation might also be the reason for increasing meat pH over time. Furthermore, it is an established fact that at room temperature, microbial load multiply as the keeping time has been lengthened, which leads to a rise in meat pH's levels. Indeed, according to Narinc *et al*. (2013), pH of quail breast meat usually declines rapidly within the first 2 h post-slaughter and inclines (5.8 - 5.9) after 4 h of aging in breast muscle. Likewise, a linear and quadratic effects were observed for temperature on day 2, while on day 3, a linear decline was observed on temperature. This was not expected under the same storage conditions, however, it could be due to climate changes experienced in different days of the storage period. Moreover, these findings can point to the high temperature, which significantly decreased the proportion of breast muscle and also the lightness on days 6 and 7 of the storage period. However, quadratic effects were observed for breast meat lightness on days 3 and 4. The higher ultimate pH value (7.08) observed for AP25 on day 7 could be an indication of low glycogen levels in the quail birds prior to slaughter. Indeed, excessive starving or malnutrition have been reported to cause high post-mortem pH values in animals (Węglarz, 2010). Therefore, lightness as an indicator for the freshness of meat was affected as a result of this higher pH, which increased the microbial load of breast meat.

Zhang and Barbut (2005), reported that breast meat with an *L** value below 46 are called to be dark, firm and dry, which means they have a dark colour, high WHC, and short shelf life. This was the case in the current study because *L** value for diet AP100 on day 7 was recorded to be 18.69. This could be due to the microbial spoilage that caused a reduction in yellowness and the increase in darker colour, in turn reducing the shelf life. In addition, quadratic effects were also observed for *a** on day 4 in response to dietary APP levels. These findings could be due to the presence of colour pigments present in AP. However, inconsistent findings were observed for the *b** value in the current study. On day 1, *b** linearly declined, while, on day 2, a positive quadratic response was recorded with increasing dietary APP levels. On day 3, *b** linearly increased with increasing dietary APP levels. This can be explained by the predominant pigments in the AP causing changes in the colour of the meat, giving it a lighter appearance (Genchev *et al*., 2008).

Currently, there is an increasing interest of consumers to quality of foods, particularly meat products that contain natural bioactive components, which would provide additional benefits to their health status (Hygreeva *et al*., 2014). The valuable taste and dietary properties of quail meat are pivotal in determining the growing interest of consumers to this product. The quality and composition of meat are influenced by numerous factors namely, the genotype of birds (Le Bihan-Duval, 2004; Genchev *et al*., 2005), feeding mode (Genchev, 2003; Genchev *et al*., 2007) and slaughtering age (Genchev *et al*., 2004). Furthermore, research has shown that quail meat production is economically effective when the birds are slaughtered at the age of 35 days (Kaitazov & Genchev, 2004). In the present study, increasing levels of APP resulted in a quadratic trend for meat CP and a linear decrease for meat EE. In breast meat samples, the quadratic trend observed for meat CP could be due to the presence of polyphenols in APP, because at lower levels they may have not been very efficient but became more pronounced as APP levels increased. An increase in CP digestibility was also observed, which could explain the increase CP content of the breast. However, excess of protein can be detrimental to the formation of muscle mass, by promoting the increased catabolism of amino acids (Zhang *et al*., 2019). This implies that too much protein in animal diets could depress muscle development and in turn affect growth performance. Nonetheless, dietary APP did not affect other meat stability traits. This could be explained by the fact that the consumption of antioxidants present in the AP could lead to the decrease in meat oxidation without altering the meat quality of the birds.

4.6 Conclusion

The inclusion of dietary apple pomace powder (APP) reduced final body and carcass weights of the quail. Similarly, dietary APP levels linearly reduced drumstick, thigh, and breast weights, and altered the sizes of small intestines and proventriculus. Storage at room temperature affected shelf-life indicators (pH, temperature, lightness, redness, and yellowness), however the inclusion of APP improved the crude protein and ether extract of breast meat composition.

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5 CHAPTER FIVE – GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Due to the growing competition for food and feed resources between animals and humans, there has been increasing interest on the use of human inedible feeds to intensify animal production (Salami *et al*., 2019). These feed resources should be characterised by their potential to provide essential nutrients and bioactive compounds without negatively affecting animal performance (Borges Soares *et al*., 2007; Alagawany *et al*., 2019). To this end, substantial amounts of agro-wastes are produced annually from agro-industrial processing companies and this waste is discarded in barren land with little regard to the environment (Bertocco Ezequiel *et al*., 2006; Di Cerbo *et al*., 2014; Alagawany *et al*., 2020). Some environmental challenges include surface and ground water pollution, foul odour, flies, and pests' attraction that may spread diseases and oxygen depletion in soil and ground waters by plant secondary compounds (Christ & Burrit, 2013; Dwyer *et al*., 2014). The use of agro-wastes such as apple pomace (AP) as a source of nutrients and bioactive substances in quail feeds could improve the birds' performance, product quality and ultimately, boost profit margins. This study, therefore, evaluated the effect of graded levels of dietary apple pomace powder (APP) on feed utilisation, growth performance, haematology, serum biochemistry, organ development, carcass characteristics, meat quality and stability parameters, and meat composition in Jumbo quail. The APP was incorporated in standard grower diet at a rate of 0 (AP0), 25 (AP25), 50 (AP50), 75 (AP75) and 100 g/kg (AP100), producing five isonitrogenous and isoenergetic diets, which were fed to 350 Jumbo quail birds.

In Chapter 3, the effect of incremental levels of dietary APP on apparent nutrient digestibility, growth performance and haemato-biochemical parameters of Jumbo quail was investigated. It was hypothesised that the inclusion of incremental levels of apple pomace powder would improve apparent nutrient digestibility, growth performance, and haematological and serum biochemical parameters in Jumbo quail.

Dietary inclusion of APP increased OM digestibility, which indicates high availability of organic nutrients for digestion and absorption by the birds. Numerically, all the other nutrients from this study decreased at the highest APP level (100 g/kg), which demonstrates that higher levels of APP could compromise feed utilisation efficiency. The decrease in the digestibility of the other nutrients could be induced by the extra dietary fibre in the APP-containing diets. It was observed that dietary inclusion of APP at 50 g/kg maximised gain-to-feed ratio (G:F) as compared to the standard control diet. Levels up to 100 g/kg were found to reduce weight gain and G:F, especially in young quail, which could be due to high fibre and polyphenolic compounds levels in APP. Serum biochemical parameter are indicators of pathophysiological state of the birds, thus their determination in Jumbo quail was meant to safeguard the wellbeing of the birds. Dietary influences were observed on alkaline phosphatase (ALKP), choline, lipase and calcium up to 50 g/kg. From the current study, quail reared on diet AP25 and AP50 had higher calcium content than the control diet. This could be attributed to the linear increase observed in the current study, which might be due to reduced feed intake in weeks 2, 3 and 4. This could have happened as a result of poor nutrients digestibility (Zanu *et al*., 2020), due to high fibrous content present in the AP. The higher concentration of serum ALKP in the quail receiving the diets AP75 and AP100 may be linked to the liver damages caused by the consumption of APP polyphenols at higher levels (AP75 and AP100), respectively (Millet *et al*., 2010). Moreover, heterophils and lymphocytes content were significantly influenced by dietary treatments. This suggests that the inclusion of APP in Jumbo quail diets might have increased calcium metabolism, which in turn result in the increase in serum calcium. This further stimulates the synthesis of bone marrow and increases the production of white blood cells, such as heterophils (Van Dijk *et al*., 2009). Since haematological and serum biochemical parameters fell within the expected normal ranges for healthy quail, it means that the inclusion of APP in quail diets did not negatively affect the health status of the birds. The hypothesis that including APP in Jumbo quail diets would improve apparent nutrient digestibility, growth performance and haemato-biochemical parameters cannot be entirely accepted, because APP inclusion only maximised G:F in week 5 at 50 g/kg.

In Chapter 4, the effect of various levels of dietary APP on carcass characteristics, internal organ development, breast meat quality and stability, and meat composition of Jumbo quail was investigated. The hypothesis tested whether the inclusion of incremental levels of apple pomace powder would enhance carcass characteristics, internal organ development, breast meat quality and stability, and meat composition of Jumbo quail. The inclusion of dietary APP resulted in linear decreases for HCW, CCW, FBW, drumstick, thigh and breast weights. Furthermore, only proventriculus was significantly influenced by dietary treatments, which was due to large accumulation fibre in APP-containing diets, which significantly resulted in poor digestibility of nutrients. However, all the other parameters were not significantly affected by dietary treatments. The results on meat quality measurements also showed a significant effect of diet on pH_{24}, lightness (L_{24}), redness (a_{24}) and $chroma_{24}$ but had no effect on cooking loss, drip loss, shear force and WHC of meat. These findings suggest that higher levels of AP (50.0 g/kg) compromised some meat quality parameters. This suggests that higher levels of APP should be used with caution in quail diets. Moreover, the significant effects on some meat quality traits indicates the potential of APP to promote normal oxidative stability for meat quality attributes during storage. Storage at room temperature affected shelf-life indicators (pH, temperature, lightness (L*), redness (a*) and yellowness (b*)). Increasing levels of APP resulted in a quadratic trend for breast meat crude protein (CP) and a linear decrease for meat ether extract (EE). Quail fed with diet AP100 displayed higher breast meat crude fibre than those fed with diets AP25 and AP75. Thus, the proposed hypothesis that feeding APP-containing diets would enhance carcass characteristics, internal organ development, breast meat quality and stability, and meat composition of Jumbo quail was not entirely accepted due to the negative responses in some of the measured parameters.

5.2 Conclusion

The results indicated that dietary inclusion of dried apple pomace powder at the rate of 50 g/kg maximized gain-to-feed ratio (G:F) but levels up to 100 g/kg reduced weight gain and G:F when compared to the standard control diet. According to the findings of this study, APP inclusion increased calcium, choline, lipase, lymphocytes, heterophils and ALKP levels up to 50 g/kg. Moreover, dietary effects were only observed on proventriculus, which indicated the accumulation of high fibre content from APP. The inclusion of dietary APP reduced carcass performance attributes of the birds. Furthermore, the findings from the current study revealed that storage effect at room temperature affected shelf-life indicators. Further, the inclusion of APP increased the crude protein and ether extract of breast meat. Overall, the inclusion of APP up to 50 g/kg has the potential to improve Jumbo quail productivity in a cost-effective, safe and sustainable manner.

5.3 Future recommendations

Reduced feed-food competition is a critical aspect of modern livestock production that will be emphasized even more in the near future due to the world's growing population. As a result, the search for alternatives to common feed ingredients is critical to the poultry industry’s long-term viability. In that regard, agro-wastes are promising candidates to supply nutrients and bioactive compounds to quail nutrition while promoting environmental sustainability. In addition, APP can be used as an alternative feed ingredient in the diet of quail birds because of its rich bioactive compounds, which can positively impact a quail meat and thus enhance satisfactory nutritional quality and consumer acceptance. However, more research is needed to improve the feed value of this by-product.

The following future studies are recommended to investigate the feed value of APP:

- To determine the effect of apple pomace powder on fatty acids profile, amino acids profile, meat sensory characteristics and technological parameters in Jumbo quail diets.
- To determine the effect of apple pomace-containing diets on oxidative stress, malondialdehyde (MDA), and lipid peroxidation of the meat in Jumbo quail.
- To determine the effect of using tannins-binding agents to ameliorate the negative effects of condensed tannins in apple pomace for Jumbo quail.
- To determine the effect of apple pomace-containing diets on histopathology, gut morphology, metagenomics, metabolomics, caecal microbiota and their metabolic pathways in Jumbo quail.
- To determine a cost-benefit analysis of apple pomace powder when included in diets of Jumbo quail.

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6 LIST OF APPENDICES

6.1 Ethics certificate

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Research Ethics Regulatory Committee
Tel: 018 299-4849
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ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC)** on 29/07/2021, the NWU Animal Production Sciences Research Ethics Committee hereby **approves** your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Feed utilisation, physiological and meat quality responses in Jumbo quail reared on apple pomace-containing diets

Study Leader/Supervisor (Principal Investigator)/Researcher: Prof K Mnisi

Student: Allen N. Matabane

Ethics number:

N	W	U	-	0	0	8	0	7	-	2	1	-	A	5
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: 2022/08/09 **Risk Category: 2**
Expiry date: 2023/08/08
Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the NWU-AnimProdREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-AnimProdREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-AnimProdREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and NWU-AnimProdREC reserves the right to:*

1

6.2 Language editing certificate

SCHOOL OF BIOLOGICAL SCIENCES

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Date: 27th November 2022

To Whom It May Concern,

REF: Language Editing and Proof-reading of Dissertations/Theses

Dear Sir or Madam,

This serves to confirm that I have proof-read and edited the **MSc dissertation** of **AN Matabane** (Student number: **27286509**; orcid.org/**0000-0001-5516-2937**) entitled: **Feed utilisation, physiological and meat quality responses in Jumbo quail reared on apple pomace-containing diets**. The candidate then later corrected all the identified language and technical errors to the satisfaction of the supervisor. Thus, the document presented here is of sufficient and acceptable academic standards.

Editor

Prof. O Ruzvidzo

Supervisor

Prof. CM Mnisi

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