

ASSESSING THE POTENTIAL OF *SPIRULINA PLATENSIS* AS A PROTEIN  
SOURCE AND ITS EFFECT ON FEED INTAKE, GROWTH PERFORMANCE,  
CARCASS YIELD, INTERNAL ORGANS SIZES AND MEAT QUALITY OF  
BOSCHVELD AND POTCHEFSTROOM KOEKOEK CHICKEN BREEDS

A THESIS SUBMITTED IN FULFILMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN  
AGRICULTURE (ANIMAL SCIENCE)

OF

THE UNIVERSITY OF NAMIBIA

BY

AINO NAMBAHU

200971981

APRIL 2024

MAIN SUPERVISOR: DR. PATRICIA N. PETRUS (UNIVERSITY OF NAMIBIA)

CO-SUPERVISOR: PROFESSOR. JOHNFISHER MUPANGWA (UNIVERSITY OF  
NAMIBIA)

## ABSTRACT

The potential of *Spirulina platensis* (SP) as a protein source to replace soybean meal and its effect on feed intake, growth performance, carcass yield, internal organ sizes and meat quality was determined. A 12 weeks feeding trial was conducted using 72 unsexed Boschveld and Potchefstroom Koekoek chickens. The chickens were fed with commercial starter crumbles from day 1 to week 4. At week 5 of age, the chickens were randomly assigned to one of four dietary treatments which were as follows; 0%SP, 5%SP, 10%SP and 15%SP of dried *Spirulina platensis* powder as a replacement of soybean meal, with six replicates per treatment and three per breed. The feeding experiment excluding brooding period lasted for 12 weeks. Feed intake data were collected daily, and live weights were taken weekly for growth performance. The results showed that feed intake responded negatively to increase in Spirulina inclusion level. Chickens on treatment 0%SP had higher weights ( $P<0.05$ ) which was not affected ( $P>0.05$ ) by the breed. The breed significantly affected ( $P<0.05$ ) average daily gain (ADG) where BV had a higher ADG compared to PK. The feed conversion ratio was highest ( $P<0.05$ ) in chickens on diet 10% SP; with a mean value of 1.03 and the lowest in 0% SP with the value of 0.98. Birds on diet 0%SP recorded the highest ( $P<0.05$ ) protein efficiency ratio PER (0.91) while those on diet 15%SP had the least PER (0.57). The results showed that dietary treatment x breed interactions did not ( $P>0.05$ ) affect the gizzard, spleen, heart, liver and intestines weights and carcass yield. However, the breed of the chicken affected the spleen size, in which Potchefstroom Koekoek chickens recorded heavy spleen weight ( $P<0.05$ ) than Boschveld. Treatment diets significantly

influenced ( $P < 0.05$ ) breast muscle and thigh weight of the chickens. The treatment diets affected ( $P < 0.05$ ) the coliform and *Lactobacillus* counts of the cecum. The coliform counts decreased significantly with an increase in Spirulina inclusion in the diet while *Lactobacillus* counts increased significantly with increasing level of Spirulina in the diet. The inclusion of Spirulina in the grower diet did not ( $P > 0.05$ ) influence the meat physiochemical quality of the chickens but compromised some eating quality parameters such as flavor and initial and sustained juiciness. A prominent chicken flavor ( $P < 0.05$ ) was recorded in chickens fed the control diet while chickens fed Spirulina had less chicken flavor. In conclusion, increasing Spirulina level in the diets negatively affected feed intake, body weights, PER, breast muscle and thigh weights and chicken flavor of the meat. Increasing Spirulina in the diets also resulted in high FCR, increased *Lactobacillus* counts and reduced coliforms. Based on these results, it is therefore best if only up to 5% Spirulina can be included in the diets for indigenous chickens. However, Spirulina was found to have high protein content but its inclusion at high levels negatively affected feed intake, meat flavor and acceptability of the meat. There is need to further investigate lower inclusion levels of *Spirulina platensis* and their effect on chicken production parameters and meat quality.

**Key words:** bacteria, body weight, breed, carcass, diet, feed intake, meat

## TABLE OF CONTENTS

ABSTRACT.....	i
List of Tables .....	viii
List of figures.....	x
List of abbreviations.....	xii
Acknowledgments.....	xiv
Dedication.....	xv
Declaration.....	xvi
<b>CHAPTER 1.....</b>	<b>1</b>
<b>INTRODUCTION.....</b>	<b>1</b>
1.1. Background to the study.....	1
1.2. Problem statement.....	4
1.3. Objectives of the study.....	5
1.3.1. General objective.....	5
1.3.2. Specific objectives.....	5
1.4. Hypotheses.....	6
1.5. Significance of the study.....	7
1.6. Delimitations of the study.....	7
1.7. Limitations of the study.....	8
1.8. References.....	9
<b>CHAPTER 2.....</b>	<b>13</b>
<b>LITERATURE RIVIEW.....</b>	<b>13</b>
2.1. Introduction.....	13
2.2. Characteristics of indigenous chickens native to Southern Africa.....	14
2.2.1. Potchefstroom Koekoek.....	14
2.2.2. Boschveld.....	15
2.2.3. Naked-neck.....	16

2.2.4. Ovambo.....	17
2.3. Protein feed resources and efficiency.....	18
2.4. An overview of <i>Spirulina platensis</i> alga.....	20
2.4.1. <i>Spirulina platensis</i> nutrient composition.....	21
2.4.2. <i>Spirulina platensis</i> secondary metabolites.....	23
2.4.2.1. Phenols.....	23
2.4.2.2. Tannins.....	23
2.4.2.3. Saponins.....	25
2.4.2.4. Alkaloids.....	25
2.5. Effects of Spirulina on growth performance of chickens.....	26
2.5.1. Effects of Spirulina on feed intake.....	26
2.5.2. Effects of Spirulina on body weight and body weight gain.....	27
2.5.3. Effects of Spirulina on feed conversion ratio.....	28
2.5.4. Effects of Spirulina on poultry gut microflora.....	29
2.6. Importance of meat proximate composition.....	31
2.6.1. Effects of Spirulina on carcass yield and internal organs.....	32
2.6.2. Effect of Spirulina on meat quality.....	34
2.7. Conclusion.....	35
2.8. References.....	37
<b>CHAPTER 3.....</b>	<b>52</b>
<b>EFFECTS OF FEEDING INCREMENTAL LEVELS OF <i>SPIRULINA PLATENSIS</i> ON FEED INTAKE AND GROWTH PERFORMANCE OF BOSHCVELD AND POTCHEFSTROOM KOEKOEK CHICKENS.....</b>	<b>52</b>
ABSTRACT.....	52
3.1. Introduction.....	54
3.2. Materials and methods.....	56
3.2.1. Bird management and care.....	56
3.2.2. Experimental design.....	57
3.2.3. Treatment diets formulation.....	57
3.2.4. Chemical composition analysis of the experimental diets.....	58

3.2.5. Anti-nutritional factors: Total phenols and tannins.....	59
3.2.6. Production parameters measurements.....	62
3.2.7. Statistical analyses.....	63
3.3. Results.....	65
3.3.1. Feed intake.....	65
3.3.2. Protein intake.....	69
3.3.3. Growth performance.....	71
3.3.3.1. Average body weights.....	71
3.3.3.2. Average daily gain (ADG).....	76
3.3.3.3. Feed conversion ratio.....	76
3.3.3.4. Protein efficiency ratio.....	77
3.4. Discussions.....	80
3.4.1. Feed intake.....	80
3.4.2. Protein intake.....	82
3.4.3. Average daily gain and average body weights.....	82
3.4.4. Feed conversion ratio.....	83
3.4.5. Protein efficiency ratio.....	84
3.5. Conclusions.....	85
3.6. Recommendations.....	85
3.7. References.....	87
<b>Chapter 4.....</b>	<b>94</b>
<b>EFFECTS OF FEEDING INCREMENTAL LEVELS OF <i>SPIRULINA PLATENSIS</i> ON CARCASS YIELD AND INTERNAL ORGAN SIZES OF BOSCHVELD AND POTCHEFSTROOM KOEKOEK CHICKEN BREEDS.....</b>	<b>94</b>
Abstract.....	94
4.1. Introduction.....	96
4.2. Materials and methods.....	98
4.2.1. Experimental design, bird management and diets.....	98
4.2.2. Slaughtering process.....	98

4.2.3. Carcass yield and internal organs size measurements.....	99
4.3. Statistical analyses.....	99
4.4 Results.....	101
4.4.1. Carcass yield.....	101
4.4.2. Internal organ sizes.....	103
4.5. Discussion.....	106
4.5.1. Carcass yield.....	106
4.5.2. Internal organ sizes.....	108
4.6. Conclusions and recommendations.....	108
4.7. References.....	110
<b>Chapter 5.....</b>	<b>114</b>
<b>EFFECTS OF FEEDING INCREMENTAL LEVELS OF <i>SPIRULINA PLATENSIS</i> ON INTESTINAL GUT MICROFLORA OF BOSCHVELD AND POTCHEFSTROOM KOEKOEK CHICKEN BREEDS.....</b>	<b>114</b>
Abstract.....	114
5.1. Introduction.....	116
5.2. Materials and methods.....	118
5.2.1. Experimental design, bird management and diets.....	118
5.2.2. Slaughtering process.....	118
5.2.3. Sample collection.....	118
5.2.4. Microbiological analysis of bird samples.....	118
5.2.4.1. Enumeration of bacteria.....	118
5.2.4.2. Isolation and bacterial identification.....	119
5.3. Statistical analyses.....	120
5.4. Results.....	121
5.4.1. Bacteria identification.....	121
5.4.2. Enumeration of gut microflora.....	122
5.5. Discussion.....	124
5.6. Conclusion and recommendations.....	125

5.7. References.....	126
<b>Chapter 6.....</b>	<b>131</b>
<b>THE EFFECTS OF FEEDING INCREMENTAL LEVELS OF <i>SPIRULINA PLATENSIS</i> ON MEAT QUALITY OF BOSCHVELD AND POTCHEFSTROOM KOEKOEK CHICKEN BREEDS.....</b>	<b>131</b>
Abstract.....	131
6.1. Introduction.....	133
6.2. Materials and methods.....	135
6.2.1. pH measurements.....	135
6.2.2. Thaw loss and cooking loss.....	135
6.2.3. Proximate analyses.....	136
6.2.4. Sensory analysis.....	136
6.2.5. Statistical analyses.....	137
6.3. Results.....	138
6.3.1. Meat quality.....	138
6.3.2. Descriptive sensory analysis and correlations.....	140
6.4. Discussion.....	145
6.4.1. Meat quality.....	145
6.4.2. Sensory analysis.....	146
6.5. Conclusions and recommendations.....	148
6.6. References.....	149
<b>Chapter 7.....</b>	<b>154</b>
<b>GENERAL CONCLUSIONS AND RECOMMENDATIONS.....</b>	<b>154</b>
7.1. Conclusions.....	154
7.2. Recommendations.....	157
<b>REFERENCES.....</b>	<b>158</b>
<b>APPENDICES.....</b>	<b>160</b>

<b>LIST OF TABLES</b>	<b>PAGE</b>
<b>Table 2.1</b> Effects of Lysine levels on protein intake, body weight gain and protein efficiency rate .....	19
<b>Table 2.2</b> Nutritional profile of <i>Spirulina platensis</i> in comparison to soybean meal.....	...22
<b>Table 2.3</b> Effects of supplementation of <i>Spirulina platensis</i> to broiler diets on feed intake and growth performance .....	28
<b>Table 2.4</b> Commercial cuts proximate composition (%) for four South African chicken breeds .....	32
<b>Table 2.5</b> Effects of <i>Spirulina platensis</i> on internal organ weight (g) in broiler chicks .....	34
<b>Table 3.1</b> Ingredients used for the formulation of grower experimental diets.....	.....58
<b>Table 3.2</b> The chemical composition and minerals content of the experimental diets, soybean meal and <i>Spirulina platensis</i> g/kg DM.....	61
<b>Table 3.3</b> The concentration of phenols and tannins (g/kg DM) in <i>Spirulina platensis</i> , soybean and experimental diets.....	62
<b>Table 3.4</b> Statistical significance of treatment diets, breed, week and their interactions on the feed intake parameters as influenced by incremental levels of <i>Spirulina</i> in the diet.....	65
<b>Table 3.5</b> Effects of treatment diets and breed interactions on feed intake parameters of Boschveld and Potchefstroom Koekoek chickens.....	66
<b>Table 3.6</b> Main effects of treatment diets and breed on feed intake parameters of Boschveld and Potchefstroom Koekoek chickens.....	67
<b>Table 3.7</b> Statistical significance of treatment diets, breed and week and their interactions on the growth performance as influenced by incremental levels of <i>Spirulina</i> in the diet.....	72
<b>Table 3.8</b> Effects of treatment diets and breed interaction on growth parameters of Boschveld and Potchefstroom Koekoek chickens.....	74

<b>Table 3.9</b> Main effects of treatment diets and breed on growth parameters of Boschveld and Potchefstroom Koekoek chickens.....	76
<b>Table 4.1</b> Effects of diet and breed interaction on carcass yield of Boschveld and Potchefstroom Koekoek chickens .....	101
<b>Table 4.2</b> Main effect of diet and breed on the carcass yield of two chicken breeds.....	103
<b>Table 4.3</b> Internal organ weights of Boschveld and Potchefstroom Koekoek chickens fed four grower diets with different inclusion levels of <i>Spirulina platensis</i> .....	104
<b>Table 4.4</b> The main effects of diet and breed on internal organ sizes as influenced by inclusion of <i>Spirulina</i> in grower diet.....	105
<b>Table 5.1</b> Cecal microbial count (cfu/g) as influenced by the interaction of chicken breeds and treatment diets.....	122
<b>Table 5.2</b> Main effect of diet and breed on cecal microbial count (cfu/g) of Boschveld and Potchefstroom Koekoek chickens.....	123
<b>Table 6.1</b> The effects of breed and dietary treatment interactions on proximate composition, thaw loss, cooking loss and meat pH as influenced by incremental levels of <i>Spirulina</i> .....	138
<b>Table 6.2</b> The effects of treatment diets and chicken breeds on proximate composition, thaw loss, cooking loss and meat pH.....	139
<b>Table 6.3</b> Statistical significance of effects of main factors (diet, breed and panelist) and their interactions on sensory attributes of chicken meat as influenced by incremental levels of <i>Spirulina</i> .....	140
<b>Table 6.4</b> Effects of incremental levels of <i>Spirulina platensis</i> on sensory attributes of two chicken breeds.....	141
<b>Table 6.5</b> Correlation coefficients (r) and p values of meat samples as influenced by inclusion of <i>Spirulina platensis</i> in grower diet at different levels.....	143
<b>Table 6.6</b> Relevant positive correlations of sensory and chemical attributes of chicken breast muscles as influenced by inclusion of <i>Spirulina platensis</i> in grower diet of chickens.....	146

## LIST OF FIGURES

### PAGE

<b>Figure 2.1</b> Potchefstroom cockerel and hen: .....	15
<b>Figure 2.2</b> Boschveld chicken breed.....	16
<b>Figure 2.3</b> Naked-neck cockerel and hens.....	17
<b>Figure 2.4</b> Ovambo cockerel and hen.....	18
<b>Figure 3.1</b> Daily feed intake per week under varying inclusion levels of Spirulina in grower diet.....	68
<b>Figure 3.2</b> Effects of breed x week interaction on feed intake of Boschveld and Potchefstroom Koekoek chickens fed incremental levels of Spirulina	69
<b>Figure 3.3</b> Effects of Spirulina platensis inclusion level on weekly protein intake (g/day) of Boschveld and Potchefstroom Koekoek chickens.....	70
<b>Figure 3.4</b> Effects of breed and week interaction on protein intake as influenced by inclusion of Spirulina in the diet.....	71
<b>Figure 3.5</b> Effects of dietary treatments and week interaction on average body weights (g) of chickens as influenced by inclusion of Spirulina in the diet .....	73
<b>Figure 3.6</b> Effects of breed and week interactions on average body weights of chickens fed incremental Spirulina level.....	75
<b>Figure 3.7</b> Effects of Spirulina platensis inclusion level on weekly protein efficiency ratio of Boschveld and Potchefstroom Koekoek chickens.....	78

<b>Figure 3.8</b> Effects of chicken breed and period of growth (week) on protein efficiency ratio.....	79
<b>Figure 5.1</b> Staphylococcus bacteria.....	121
<b>Figure 5.2</b> Lactobacillus bacteria.....	121
<b>Figure 5.3</b> Coliforms.....	121
<b>Figure 6.1</b> Mean points for sensory attributes per treatment diets as influenced by incremental levels of Spirulina.....	144

## LIST OF ABBREVIATIONS AND ACRONYMS

AA	Amino acids
ADF	Acid Detergent Fiber
ADG	Average daily gain
ANOVA	Analysis of Variance
ANFs	Anti-nutritional factors
AOAC	Association of Official Analytical Chemists International
BIC	Bayesian Information Criterion
BV	Boschveld
Ca	Calcium
CFU	Colony forming units
CP	Crude protein
CRD	Complete randomized design
CS	Compound symmetry
CT	Condensed tannins
Cu	Copper
DAFF	Department of Agriculture, Forestry and Fisheries
DM	Dry matter
EDTA	Ethylenediaminetetraacetic acid
EE	Ether extract
FCR	Feed Conversion Ratio
Fe	Iron
GLM	General linear model
HT	Hydrolyzable tannins
HCS	Heterogeneous compound symmetry
HF	Huynh- Feldt
IB	Infectious Bronchitis

K	Potassium
LAB	<i>Lactobacillus</i>
Mg	Magnesium
Mn	Manganese
MRS	De Man-Rogosa- Sharpe
Na	Sodium
NDF	Nitrogen detergent fiber
OM	Organic matter
PER	Protein Efficiency Ratio
PK	Potchefstroom Koekoek
PSE	Pale soft exudate
SP	Spirulina
SPSS	Statistical package for social sciences
TNTC	Too numerous to count
TLTC	Too little to count
TP	Total phenols
TT	Total tannins
VC	Variance components
Zn	Zinc

## ACKNOWLEDGEMENTS

I am very thankful to my supervisors Dr. Petrus Patricia and Professor Johnfisher Mupangwa for their guidance from the very beginning of developing this research topic to its implementation. I thank you all for your support, motivation and valuable inputs of this thesis. I acknowledge the help and guidance of Dr. Vonai Charamba and Dr. Emmanuel Lutaaya for their guidance and help with data analysis.

I would further recognise the Brandenburg University of Technology, Germany, for their support in supplying the Spirulina that was used in this research. Your support is very much appreciated.

The following people deserves a special recognition for helping me out throughout my feeding experiment and laboratory work; Johannes Phillipus, Natalia Moses, Joyce Mwazi, Samuel Hangula, Eilzabeth Nambahu and Magdalena Kamati, without your assistance I would not have managed on my own. May the almighty bless you more and continue doing well to others as you did to me.

To the University of Namibia and all my colleagues in the Department of Animal Production, Agribusiness and Economics, thank you all for your support.

Finally, I give thanks to the Almighty God who made everything possible!

## **DEDICATION**

This thesis is dedicated to my lovely late grandparents, Mr. Iivali Matias (M̄ solotati) and Mrs. Emilia Titus (Gwakawali). I cannot thank you enough for your unmatched love and care. I could not be whom I am today if it was not for you. I miss you all dearly, may your souls continue resting in peace.

## DECLARATION

I Aino Nambahu hereby declare that this is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution. No part of this thesis may be reproduced, stored in any retrieval system, or transmitted in any form, or by means (e.g. electronic, mechanical, photocopying, recording or otherwise) without the prior permission of the author, or The University of Namibia in that behalf.

I Aino Nambahu, grant The University of Namibia the right to reproduce this thesis in whole or in part, in any manner or format, which The University of Namibia may deem fit.

Aino Nambahu	.....	April 2024
Name of student	Signature	Date

## CHAPTER 1

### INTRODUCTION

#### 1.1. Background to the study

Poultry production is of enormous importance in the livelihoods of many rural communities in the developing countries especially the indigenous chickens (Melesse, 2014). According to Sonaiya (2007) indigenous chickens (*Gallus domesticus*) are valuable and have a very significant position in livelihood of rural communities. In addition, farmers in the resource-poor communities prefer rearing indigenous chickens as compared to exotic chickens due to their high adaptability to the environment which is linked to these chicken`s genetic potential to thrive in their production systems and environment (Masaire *et al.*, 2018). Indigenous chickens are well preferred by the rural communities where they are reared under extensive system of production (Sebola *et al.*, 2015). However, indigenous chickens of Southern Africa such as Boschveld, Potchefstroom Koekoek, Naked-neck and Ovambo, are less competitive when compared to exotic and commercial breeds due to their slow growth and low production which is mainly related to their low genetic potential, lack of proper nutrition and their vulnerability to disease outbreaks because they do not receive prophylactic medications (Magothe *et al.*, 2012).

The poultry production systems in rural Africa are merely centered around subsistence production of which the chickens are raised as scavengers, and they less taken care of in terms of housing, health care and feeding (Akinola & Essien, 2011). Farmers depend

on hens to perform hatching and taking care of chicks, which causes long reproductive cycles and high chicks death. Nevertheless, indigenous chickens add significant contribution to the livelihood of rural farmers especially women through provision of high quality animal protein from the consumption of meat and eggs (Petrus, 2011). Furthermore, indigenous chickens through sales of meat, eggs, live birds and manure also provide family income which is used to purchase essential commodities and pay for services such as school fees and hospital services (Petrus, 2011).

There are many challenges to poultry production of which the main challenge is poor nutrition in terms of both quality and quantity of feed and poultry health (Masaire *et al.*, 2018). The rising cost of conventional feed ingredients such as, maize, wheat and soybean meal due to competition for food, feed, biofuel and bioprocessing industries has presented an opportunity for nutritionist to research other possible feed ingredients that can be explored as protein sources for animals (Babatunde *et al.*, 2021). The cause of these challenges is mainly associated with climate change which has been major problem affecting rainfall which in turns affects water availability and animal feeds in most countries (Bagopi *et al.*, 2014). As a result, poultry farmers face the challenge of finding a protein source for poultry which can adapt to the current climate and do not compete for crop production land and water. It is important to identify alternative feed ingredients or resources to sustain the production of animals and most importantly to potentially improve the production of indigenous chickens (Packard, 2014). Significantly, the feed resources to be identified must have high nutrient content and high feed conversion ratio, capable to use land and water efficiently and overall, it should result in an animal product that is of good quality and acceptable by consumers

(Poppi & McLennan, 2010). Therefore, it is crucial to explore other source of functional ingredients for example exploring algae and more especially micro algae (i.e. *Spirulina platensis*) as alternative protein source (Lordan *et al.*, 2011; Draaisma *et al.*, 2013).

According to Prashantkurmar *et al.* (2006) algae have demonstrated the potential to be used as alternative protein source for humans and animals with special interest on *Spirulina platensis*. *Spirulina platensis* is a cyanobacteria that is considered to have natural antioxidant compounds such as flavonoids, alkaloids, phenols and steroids (Nagy, 2020). Due to its high amount of protein it can be explored as an alternative protein ingredient for poultry feed (Gongnet *et al.*, 2001). *Spirulina* has high proteins of 50-70% (Anbarasan *et al.*, 2011). *Spirulina* has good percentage of essential amino acids, minerals, good pigment and vitamins, it is rich in polysaccharides which makes it a good prebiotic (Sugiharto *et al.*, 2017). *Spirulina* demonstrated a potential to be used as another dietary ingredient for the formulation of diets that need higher amount of essential amino acids and metabolizable energy. Additionally, *Spirulina* can replace up to 15% of broiler diets as partial replacement of common protein sources without causing detrimental effect on production performance (Moustafa *et al.*, 2021). When algae are used even in small amount they may improve the gut microflora, immune function and the metabolism of lipids, immune system, increase desire for food, size and amount of eggs produced and reproductive performance (Hajati & Zaghari, 2019). In poultry, algae are found to be able to replace partially common protein sources and may also be used as almost the only protein source in layers and they are approved as chicken feed in many countries (Kovac *et al.*, 2013; Becker, 2007).

Feeding indigenous chickens remains a challenge to many farmers in the rural areas and there is a need to find alternative feed resources in order to reduce the feed challenges currently faced by the rural communities. Replacements are considered as necessary in order to reduce the high costs of importing soybean meal and decrease the protein gap (Altmann *et al.*, 2018). With many studies focusing on the incorporation of *Spirulina* as a protein source in feeds for broiler chickens, there are no studies on the application of *Spirulina* as feed ingredient for indigenous or dual purpose chickens. Therefore, as a stepping stone to previous studies, this study aims to assess the effect of *Spirulina platensis* on the feed intake, growth performance, carcass yield, gut microflora and meat quality of Boschveld and Potchefstroom Koekoek chicken breeds.

## **1.2. Problem statement**

Poultry farming in many rural areas in Namibia is characterized by use of indigenous chickens and scavenging operations. Chickens are mainly raised on crop by products such as pearl millet bran and maize bran with few being offered pearl millet grains as their daily feed. With maize and soybean being the only two main conventional feedstuffs for animal feeding, it was realized that the current allocations of maize and soybean is unsustainable since Namibia`s climate is not ideal for expansive maize and soya operations (Bagopi *et al.*, 2014). Due to recent change in climate, it has however not spare rural farmers that are mainly surviving on crop production, which affected indigenous chicken production as there is little to no food available for domesticated fowls. Additionally, the growth for human and livestock population has increased the competition for food between animals and man. This has led to the search for possible

feed resources that have high amount of nutrients and requires little to no land, and that can be grown using technologies that do not necessarily depend on rainfall. The use of *Spirulina platensis* in feed production of indigenous chickens being understudied and the focus has mostly being directed to broiler chickens, it is therefore crucial to also consider indigenous chickens in the search for feed ingredients that will possible improve their growth and production and to maintain the balance among food, feed and biofuel industries (Madeira *et al.*, 2017). This is because it is essential to increase the productivity of indigenous chickens, their growth performance and meat quality through proper feeding and housing (Petrus, 2011).

### **1.3.Objectives of the study**

#### **1.3.1. General objective**

The main objective of the study was to determine the effect of feeding incremental levels of *Spirulina platensis* as a protein source on the feed intake, growth performance, intestinal gut microflora, carcass yield and meat quality in Boschveld and Potchefstroom Koekoek chicken breeds.

#### **1.3.2. Specific objectives**

- 1.** To determine the effect of feeding graded levels of *Spirulina platensis* on feed intake and growth performance of Boschveld and Potchefstroom Koekoek chickens.

2. To determine the effect of feeding incremental levels of *Spirulina platensis* as replacement for soybean meal on carcass cuts yield and internal organ sizes of Boschveld and Potchefstroom Koekoek chickens.
3. To determine the effect of feeding incremental levels of *Spirulina platensis* as replacement for soybean meal on intestinal gut microflora of Boschveld and Potchefstroom Koekoek chickens.
4. To determine the effect of feeding incremental levels of *Spirulina platensis* as replacement for soybean meal on meat quality and sensory attributes of Boschveld and Potchefstroom Koekoek chickens.

#### **1.4. Hypotheses**

**H<sub>0</sub>:** Feeding graded levels of *Spirulina platensis* does not influence the feed intake and growth performance of Boschveld and Potchefstroom Koekoek chickens.

**H<sub>0</sub>:** Feeding incremental levels of *Spirulina platensis* does not influence the carcass cuts yield and internal organ sizes of Boschveld and Potchefstroom Koekoek chickens.

**H<sub>0</sub>:** Feeding incremental levels of *Spirulina platensis* does not influence intestinal gut microflora of Boschveld and Potchefstroom Koekoek chickens.

**H<sub>0</sub>:** Feeding incremental levels of *Spirulina platensis* does not influence the meat quality and sensory attributes of Boschveld and Potchefstroom Koekoek chickens.

### **1.5. Significance of the study**

The study will provide baseline information on *Spirulina platensis* nutritional composition and its potential as a protein source in indigenous chicken feeds. The results will provide information on the possibility of *Spirulina platensis* as a replacement for soybean in indigenous chicken feed rations. The results will also give an idea on the ideal level of Spirulina to be used in chicken feed formulation. The study results will provide nutritional information to the consumers of the chicken meat from the two breeds Boschveld and Potchefstroom Koekoek. Finally, the study results may create a new feed opportunity for local poultry feed manufacturers. This would also provide an opportunity for algae biomass production in Namibia that would create job opportunities.

### **1.6. Delimitations of the study**

The study was limited to the use of *Spirulina platensis* as a protein source and assessing its potential at four replacement levels of Soybean meal in grower diet of dual-purpose chickens. The study also focused only on two chicken breeds (Boschveld and Potchefstroom Koekoek). The parameters assessed were limited to feed intake, growth performance, intestinal gut microflora, carcass cuts yield, internal organ sizes and meat microbial load and quality. The proximate analyses done were limited by the type of analysis possible during the time of research study and the cost of analyses.

### **1.7.Limitations of the study**

The unavailability of some equipment, expertise and reagents limited the analyses of some important nutrient analysis that were necessary for the study such as fatty acids analysis and intestinal morphology. The use of cages on the chickens from a very young (5 weeks old) caused challenges with the birds as the chickens started experiencing leg deformities, which resulted in the experiment ending earlier than planned due to animal welfare issues.

## 1.8. References

- Akinola, L. A. F., & Essien, A. (2011). Relevance of rural poultry production in developing countries with special reference to Africa. *World's Poultry Science Journal*, 67: 697-705.
- Altmann, B. A., Neumann, C., Velten, S., Liebert, F., & Mörlein, D. (2018). Meat quality derived from high inclusion of a micro-alga or insect meal as an alternative protein source in poultry diets: A pilot study. *Foods*, 7: 34.
- Anbarasan, V., Kumar, V. K., Kumar, P. S., & Venkatachalam, T. (2011). In vitro evaluation of antioxidant activity of blue green algae *Spirulina platensis*. *International Journal of Pharmaceutical Sciences and Research*, 2(10), 2616.
- Babatunde, O. O., Park, C. S., & Adeola, O. (2021). Nutritional potentials of atypical feed ingredients for broiler chickens and pigs. *Animals*, 11(5), 1196.
- Bagopi, E., Chokwe, E., Halse, P., Hausiku, J., Humavindu, M., Kalapula, W., & Roberts, S. (2016). Competition, agro-processing and regional development: The case of the poultry section in South Africa, Botswana, Namibia and Zambia. *Competition in Africa: Insights from Key Industries*, 66-101.
- Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*. 25: 207-210.
- Draaisma R., Wijffels R., Slegers P., & Brentner L. (2013). Food commodities from microalgae. *Current Option in Biotechnology*, 24: 169-177.

- Gongnet, G. P., Niess, E., Rodehutsord, M., & Pfeffer, E. (2001). Algae-meal (*Spirulina platensis*) from lake Chad replacing soybean-meal in broiler diets. *Archiv Fur Geflugelkunde*, 65 (6): 265-268.
- Hajati, H., & Zaghari, M. (2019). *Spirulina Platensis in poultry nutrition*. Cambridge Scholars Publishing.
- Kovač, D., Simeunović, J., Babić, O., Mišan, A. Č., & Milovanović, I. L. (2013). Algae in food and feed. *Food and Feed Research*, 40(1): 21-31.
- Lordan S., Ross R. P., & Stanton C. (2011). Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic. *Marine Drugs*, 9: 1056-1100.
- Madeira, M. S., Cardoso, C., Lopes, P. A., Coelho, D., & Afonso, C. (2017). Microalgae as feed ingredients for livestock production and meat quality : A review. *Livestock Science*, 205: 111–121.
- Magothe, T. M., Okeno, T. O., Muhuyi, W. B., & Kahi, A. K. (2012). Indigenous chicken production in Kenya : II . *Prospects for Research and Development*, 68: 133–145.
- Masaire, E., Madzingira, O., Samkange, A., Kandiwa, E., Mushonga, B., & As, B. (2018). Characterization of poultry production and management systems in the communal areas of Namibia. *African Journal of Poultry Farming*, 6(3): 265–276.
- Melesse, A. (2014). Significance of scavenging chicken production in the ruralcommunity of Africa for enhanced food security. *World`s Poultry Science*

*Journal*, 70: 593-606.

- Moustafa, E. S., Alsanie, W. F., Gaber, A., Kamel, N. N., Alaqil, A. A., & Abbas, A. O. (2021). Blue-green algae (*Spirulina platensis*) alleviates the negative impact of heat stress on broiler production performance and redox status. *Animals*, 11(5), 1243.
- Nagy, W. M. (2020). Role of *Spirulina Platensis* as a Natural Antioxidant on in Vivo and In Vitro Embryo Development in Rabbits. *Journal of Animal and Poultry Production*, 11(7), 271-275.
- Packard, R. (2014). *Comparison of production parameters and meat quality characteristics of South African indigenous chickens* (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Petrus, N. P. (2011). *Characterisation and production performance of indigenous chickens in Northern Namibia regions* (Doctoral dissertation: University of Namibia).
- Poppi, D. P., & McLennan, S. R. (2010). Nutritional research to meet future challenges. *Animal Production Science*, 50: 329-338.
- Prashantkurmar, P., Angadi, S.B., & Vidyasagar, G. M. (2006). Antimicrobial activity of blue-green and green algae. *Indian Journal of Pharmaceutical Sciences*, 68(5): 647-648.
- Sebola, N. A., Mlambo, V., Mokoboki, H.K., & Muchenje, V. (2015). Growth performance and carcass characteristics of three chicken strains in response to incremental levels of dietary *Moringa oleifera* leaf meal. *Livestock Science*,

178:202-208.

Sonaiya, E. B. (2007). Family poultry, food security and the impact of HPAI. *World`s Poultry Science Journal*, 63(1): 132-138.

Sugiharto S, Atmaja BM, Widiastuti E, Hadiyanto H. (2021). Combined use of *Spirulina platensis* and *Saccharomyces cerevisiae*: Implication on growth, blood profile and intestinal morphology and bacteria of the Indonesian crossbred chickens. *Biodiversitas*, 23: 160-1

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1.Introduction

Generally, indigenous chickens are conserved in Africa for a long in subsistence systems (Menge *et al.*, 2005). Moreover, the indigenous chickens are also known as traditional, scavenging, backyard, village, local, marathon or family chickens. In many countries especially in Africa and rural Asia, poultry farming is mostly founded on indigenous chicken breeds and backyard operations systems (Kingori *et al.*, 2007). In addition, research has shown that there is an increase in the demand for meat, eggs, and other products from animals due to a change in preferences by consumer which is attributed to increase in wealth of these consumers and the growth of the markets, more especially in developing countries (Myers & Kent, 2003). Most important of all, the current consumers are demanding organic products that are safe to eat and can contribute to health benefits with their nutritional contents while limiting the risk of chronic diseases (Plaza *et al.*, 2009).

However, there are problems that need to be overcome first before these growing demands can be met. Firstly, is the growing competition for agricultural land and for urban and rural development, and secondly is the climate change that has been a major problem affecting the availability of water and consequently the availability of animal feeds in production regions. The world is revolving around food and feed making, sustainability of the environment, health and economic growth (Rajauria *et al.*, 2015)

## **2.2.Characteristics of indigenous chickens native to Southern Africa**

According to Choe *et al.* (2010) indigenous chickens are commonly found in the rural areas. Backyard poultry production is on the rise in the urban areas, despite this fact; the increase in the demand for chicken meat is largely catered for by rapid growth of commercial meat breeds with less contributions from local breeds. Among the indigenous chicken breeds of Southern Africa are: Potchefstroom Koekoek, Naked Neck, Venda, Boschveld and Ovambo (Packard, 2014; Motsepe *et al.*, 2016). Indigenous chickens and commercial broilers differs genetically which affects their growth rate, which is linked to the breed of the chicken that is known to affect growth performance at different ages (Selaledi *et al.*, 2021). Nonetheless, farming of indigenous chickens in rural areas plays a role in poverty mitigation and their consumption is related with less health threats (Motsepe *et al.*, 2016).

### **2.2.1. Potchefstroom Koekoek**

Potchefstroom Koekoek was developed at the Institute of Animal Husbandry and Dairying in Potchefstroom by Chris Marais in 1950s (Anonymous, 2022). The line was developed from crossing a Black Australorp, White Leghorns and Plymouth Rocks. It was given its name based on the patterns of its color, the Koekoek has a characteristic of black and white specked plumage (Figure. 2.1). The color and patterns of its feathers is gender-linked and it matures at 130 days with average weight around 3-4kg for males and 2.1 kg for female (Manyelo *et al.*, 2020). The breed is popular amongst South African farmers and farmers in the neighboring countries including Namibia. Farmers prefer this type of breed because for its egg production, meat production as well as its hen`s ability to brood her eggs and take care her chicks.



**Figure.2.1** Potchefstroom cockerel and hen. Source: Own picture

### **2.2.2. Boschveld**

Boschveld chicken is a synthetic African indigenous breed which was bred at the Mantsole ranch in the Limpopo province of South Africa in 1998. The Boschveld chicken is a cross from three indigenous lines of Southern Africa (Venda, Ovambo and Matabele chickens). Boschveld chickens have a light red-brown color with white feathers in-between (Figure. 2.2) to help and protect them from predators through camouflage (Anonymous, 2022). The Boschveld chickens were specifically bred to produce meat and eggs under harsh environmental conditions. This breed is well known for its for good mothering ability and it weighs about 2.5-3kg (Manyelo *et al.*, 2020). The breed is also found in Namibia of which it was imported and is now a common breed

in Namibia which is raised in mostly in backyard poultry farming (Petrus *et al.*, 2019).



**Figure. 2.2** Boschveld chicken breed. Source: Manyelo *et al.*, (2020)

### **2.2.3. Naked-neck**

The breed was introduced to Africa by traders from Malaysia. Naked-Neck carries a major gene  $Na-$  for Naked Neck which causes the ‘naked neck’ phenotype’. The distinctive characteristic of this line is a hairless neck and also the crop area of the breast (Figure 2.3). The chickens from this line that are homozygous for the  $Na-$  gene display a bald neck whilst heterozygous birds have a tuft of feathers on the lower part of the neck (Packard, 2014). Moreover, the Naked-Neck chickens are very colorful- white, red and black feather combinations. The egg color varies from cream to light brown (Van-Marle-Koster, 2001). Furthermore, the breed has increased weight gain, dressing percentage; it is adapted to high environmental temperatures and

resistant against most diseases (Manyelo *et al.*, 2020).



**Figure 2.3** Naked- neck cockerel and hens. Source: Packard (2014)

#### **2.2.4. Ovambo**

The Ovambo breed is a typical line well-known by the local Aawambo people as a breed of chicken inherent to the area of Ovamboland in the north central areas of Namibia; hence the name Ovambo which refers to the geographical area where they were first distinguished (Van- Marle Koster, 2001). The breed is lighter as compared to Potchefstroom Koekoek and it is well-known for its resistance to diseases (Motsepe *et al.*, 2016). The roosters weigh between 1.7-2.1 kg and hens 1.24-1.4 kg (Packard, 2014). The feathers are mostly dark red, brown and black, they also have a smaller body size which camouflage it and protect it from predators (Figure 2.4). This chicken line tends to be aggressive especially when their offspring are in danger, they often roost in trees to avoid predators (Van- Marle Koster, 2001). They have high meat and

egg production (Manyelo *et al.*, 2020).



**Figure 2.4** Ovambo cockerel and hen. Source: Grobbelaar *et al.* (2010)

### **2.3. Protein feed resources and efficiency**

Younas and Yaqoob (2005) defined poultry feed resources as any feed component that is edible by chickens and with reasonable amount of nutrients. Moreover, the most important nutrient content of a feed component are energy and protein (Younas & Yaqoob, 2005). Plants and animal products are utilized in poultry feeds as source of protein, with protein from animals being regarded as of good quality compared to the protein from plants (Ravindran, 2013). Protein can also be obtained from other source of feeds. Above all, the most commonly energy and protein source used worldwide is maize and soybean, respectively, while cereals such as wheat and sorghum and other protein feed ingredients are used as well across the world. Nevertheless, soybean meal is the most preferred protein source used in poultry feed manufacturing, certainly because

soybean protein have well balance amino acids profile and its crude protein is about 40-48% (Ravindran, 2013).

Other sources of poultry protein are insects, fly larvae, earthworms, termites, bees, snails and skimmed milk powder. According to Van Krimpen *et al.* (2013), seaweed and microalgae are also utilized as poultry feeds due their higher protein yield per unit area as compared to terrestrial crops such as soybean, pulse legumes, and wheat. Diets formulated with high lysine level 120% (National Research Council, 1994) promote a better conversion of amino acids (AA) into body weight gain. Furthermore, differences in dietary AA density responses to dietary AA/CP density differs among strain sources (Nasr *et al.*, 2011). Muscle development is mostly correlated to the amount of nuclei or total DNA (Nasr & Kheiri, 2012), therefore, strains showing fast muscle development should have balanced dietary AA which is needed for muscle growth. Therefore, a low protein diets offered to meat chickens could result in reduced growth performance (Saleh *et al.*, 2021). Protein efficiency ratio differs with the level of lysine (Table. 2.1).

**Table 2.1** Effects of Lysine levels on protein intake, body weight gain and protein efficiency rate ( Source: Nasr *et al.*, 2011)

<b>Lysine levels %</b>	<b>Protein intake (g) 0-42 days</b>	<b>Body weight gain (g) 0-42 days</b>	<b>Protein efficiency rate 0-42 days</b>
120% NRC	865.300	2227.00	2.520
110% NRC	788.700	2015.00	2.500
100 % NRC	756.600	1979.00	2.560
90 % NRC	771.400	1925.00	2.440

#### **2.4.An overview of *Spirulina platensis* algae**

Microalgae known as phytoplankton includes unicellular organisms that resides in water, they known as source of food for most aquatic fauna and causing seasonal algal blooms in rivers, lakes and ponds under eutrophic conditions (Rajauria *et al.*, 2015). Micro algae are able to grow under different environmental conditions (Hamouda *et al.*, 2022). *Spirulina platensis* is a micro algae which is excellent at producing oxygen through photosynthesis (Cavalier-Smith, 2007).

*Spirulina platensis* blue-green in color and takes the form of very small green filaments, measuring 0.1 mm in diameter, coiled in number depending on the strain and it can grow naturally in the alkaline waters of lakes in warm regions (Saranraj & Sivasakthi, 2014). *Spirulina platensis* is widely distributed in Africa, Asia and South America (Vonshak *et al.*, 2000). *Spirulina* is known to absorb a lot of carbon dioxide (CO<sub>2</sub>) during its production which contributes to the potential of *Spirulina* being able to capture CO<sub>2</sub> and reduces the carbon footprint and this makes *Spirulina* production a good way of combating global warming (Prinajati, 2021). Several studies have set to consider *Spirulina* as a panted non-antibiotic replacement as animal feed supplement owing it to its ability to promote growth and its antioxidant effect on most species especially poultry (Alvarenga *et al.*, 2011; Thormar, 2012; Wang *et al.*, 2013; Abdel-Daim, 2014). Recently, it has been concluded that inclusion of *Spirulina* in chicken feeds improved body weight gain, feed conversion ratio and improves intestinal morphology by increasing the height of the intestine`s villi (Kharde *et al.*, 2012; Shanmugapriya *et al.*, 2015; Park *et al.*, 2018).

#### **2.4.1. *Spirulina platensis* nutrient composition**

*Spirulina platensis* is rich in nutrients such as protein and essential amino acids, and has good prebiotics properties (Sugiharto *et al.*, 2022). It contains lipids (5-6%) and have most of the essential fatty acids (Anbarasan *et al.*, 2011). *Spirulina* has a good nutritional profile as compared to soya bean meal (Table.2.2), hence, incorporation of *Spirulina* in the animal diet is likely to promote the growth of lactobacillus bacteria and improve the absorption of dietary vitamins, minerals, amino acids and other nutrients (Mariey *et al.*, 2012).

**Table. 2.2** Nutritional profile of *Spirulina platensis* in comparison to soybean meal

	<i>Spirulina platensis</i>	Soybean meal
<b>Proximate composition (g 100g<sup>-1</sup> as is)</b>		
Dry matter	82.2 <sup>a</sup>	92.09 <sup>b</sup>
Crude protein	61.3 <sup>a</sup>	31.14 <sup>b</sup>
Ash	6.9 <sup>a</sup>	4.93 <sup>b</sup>
Crude fiber	3.0 <sup>a</sup>	8.84 <sup>b</sup>
Ether Extract	0.63 <sup>c</sup>	7.23 <sup>b</sup>
Energy (Mj/kg)	14.9 <sup>a</sup>	18.43 <sup>b</sup>
<b>Essential amino acids (g100g<sup>-1</sup>)</b>		
Arginine	4.10 <sup>a</sup>	3.48 <sup>c</sup>
Lysine	3.10 <sup>a</sup>	2.96 <sup>c</sup>
Isoleucine	2.60 <sup>a</sup>	2.12 <sup>c</sup>
Leucine	4.70 <sup>a</sup>	3.74 <sup>c</sup>
Histidine	1.00 <sup>a</sup>	1.28 <sup>c</sup>
Methionine	1.37 <sup>a</sup>	0.67 <sup>c</sup>
Phenylalanine	2.50 <sup>a</sup>	2.34 <sup>c</sup>
Threonine	2.70 <sup>a</sup>	0.82 <sup>c</sup>
Tryptophan	1.20 <sup>a</sup>	0.74 <sup>c</sup>
Valine	0.30 <sup>a</sup>	2.22 <sup>c</sup>
<b>Mineral content per 100g</b>		
Calcium	1.2 <sup>d</sup>	0.27 <sup>c</sup>
Total Phosphorus	13.0 <sup>d</sup>	0.62 <sup>c</sup>

Source: <sup>a</sup> Sarker *et al.*, (2016) ; <sup>b</sup> Khalila *et al.*, (2019); <sup>c</sup> National Research Council, (1994); <sup>d</sup> Yusuf *et al.*, (2016), <sup>e</sup> Zahir *et al.*, (2019)

#### **2.4.2. *Spirulina platensis* secondary metabolites**

Secondary metabolites also known as anti-nutritional factors (ANFs) are described as chemical compounds that are produced by the plant cell through metabolic pathways derived from primary metabolic pathways (Hussein & El-Anssary, 2019). Secondary metabolism product includes phenolics which are further classified into; tannins, alkaloids, flavonoids, saponins, steroids, quinines, cumarins, glycosides, triterpenoids (Hussein & El-Anssary, 2019).

##### **2.4.2.1. Phenols**

Phenolics are compounds distinguished by a benzene ring consisting of one or more hydroxyl group (Dykes & Rooney, 2007). Phenolics have beneficial properties while other phenolics such as tannins, saponins and toxic alkaloids have anti-nutritional properties in animal diets (Mahfuz *et al.*, 2021). When phenolic compound with anti-nutritional properties are present in animal feed they reduce the bioavailability of nutrients by interfering with their digestion and metabolism (Gxasheka *et al.*, 2015). Consequently, it results in decreased overall digestibility, poor feed palatability, reduced feed intake and low growth rate (Tadelle, 2015; Yacout, 2016).

##### **2.4.2.2. Tannins**

Tannins are a group of phenolics which belongs to the secondary metabolites synthesized by plants and microorganisms to archive their ecological functions (Hassan *et al.*, 2020; Kumar *et al.*, 2022). Additionally, tannins vary in concentration within different species of plants and they behave as anti-nutrients which is a result of their

capability to reduce food digestibility by binding to salivary protein and digestive enzymes leading to protein disruption (Rashwan & Hammad, 2020). Tannins are divided in two groups; hydrolysable and condensed tannins depending on their physical and chemical properties (Gxasheka *et al.*, 2015). Hydrolysable tannins can be hydrolyzed by acids or enzymes while condensed tannins are non-hydrolyzable oligomeric and polymeric proanthocyanidins (Hassan *et al.*, 2020).

Tannins can be of benefits but can also be dangerous to animals because of their toxicity but this is influenced the type of tannins and the concentration, as well as the type and age of animal (Shiningavamwe, 2022). Utilization of tannins in monogastric has not been recommended in the past due to their anti-nutritional properties (Hassan *et al.*, 2020). However, the utilization of tannins with caution has been found to benefit animals and some current studies has found the use of tannins in poultry to have favorable outcomes (Hassan *et al.*, 2020). The process through which tannins promote growth in monogastric animals are not well documented as in ruminants. Several literatures on effect of tannins on poultry have different findings with some noting that poultry are more sensitive to tannins (Suleyman, 2017), while others are suggesting that low concentration of tannins inclusion in feed results in increased feed intake and performance of monogastric animals (Hassan *et al.*, 2020). High amount of condensed tannins in animal feed can precipitate protein digestion which leads to reduced performance in monogastric animals, hydrolysable tannins are reported to improve health status of chickens offered (Mahfuz *et al.*, 2021).

Several studies have been conducted on the phytochemical properties of *Spirulina platensis*, the study's results show the presence of tannins in *Spirulina platensis* powder

(El Din *et al.*, 2019; Hamouda *et al.*, 2022). Qualitative and quantitative analysis of *Spirulina platensis* extracts revealed a low tannin content while gallic tannins were also found to be in little amount. These results however revealed that *Spirulina* has no important amount of tannins (Ennaji *et al.*, 2021; Kumar *et al.*, 2022).

#### **2.4.2.3.Saponins**

Saponins are glycosides which are secondary metabolites distinguished by bitter taste, foaming in aqueous solution and haemolyse red blood cells (Kumar *et al.*, 2009; Wang *et al.*, 2022). According to Ramteke *et al.* (2019), poultry are more susceptible to saponins than pigs. Furthermore, 0.4-0.5% saponins in the feed reduces feed intake which results in low body weight and decreases egg production (Ramteke *et al.*, 2019). Saponins are amongst plant compounds which have beneficial effects such as antibacterial and anti-protozoal properties (Ramteke *et al.*, 2019). Dried *Spirulina* powder show positive results for the presence of saponins (Agustini *et al.*, 2015; El Din *et al.*, 2019).

#### **2.4.2.4.Alkaloids**

Alkaloids are a group of nitrogen-containing compound that is naturally found as secondary metabolite (Chukwuebuka & Chinenye, 2015). They are readily available in leaves, bark, roots or seed of plants and they have diverse functions, although they act on a diversity of metabolic systems, they almost uniformly invoke bitter taste (Teferedegne, 2000). Thus, their presence in the feed worsen the taste properties of the feed and reduces feed intake which slows down the growth rate and performance

(Matsuura & Fett-Neto, 2015). Alkaloids can inhibit the activity of acetylcholinesterase, an enzyme that catalyzes the breakdown of acetylcholine that function as neurotransmitters which makes them involved in neurotoxicity (Lo *et al.*, 2018). Additionally, consumption of tropane alkaloids may lead to fatalities due to rapid heartbeat and paralysis, while muscarine alkaloids can cause acute necrosis of liver and death (Chukwuebuka & Chinenye, 2015).

## **2.5. Effects of Spirulina on growth performance of chickens**

### **2.5.1. Effects of Spirulina on feed intake**

Feed consumption is the main factor that influences both the body weight gain and feed efficiency in poultry production (Ferket & Gernat, 2006). However, voluntary feed intake is affected by many factors such as age, strain, palatability of feed, accessibility of feed, flock density, bird health status, ambient environment and nutrient balance of the diet (Cuc *et al.*, 2006). The main factor affecting voluntary feed intake is the nutrient balance of the diet. In addition to appropriate feed formulation, maintaining high feed intake is the most important factor that will determine the growth rate and efficiency of nutrient utilization in poultry (Cuc *et al.*, 2006).

Sugiharto *et al.* (2022) reported that there was no significant difference in feed intake for the entire periods between birds supplemented with Spirulina and the control group. Mirzaie *et al.* (2017) & Pestana *et al.* (2020) also got the same results of which the addition of Spirulina supplement did not have a varying effect on feed intake of the broiler chickens. On the other hand, Gongnet *et al.* (2001) reported that feed intake was reduced with increasing concentration of Spirulina meal, slightly in birds fed diet 50g/kg

and significantly in birds fed diets 100g/kg and 150g/kg. In contrast, Khan *et al.* (2013) reported an increased feed intake for birds supplemented with 2g/kg of Spirulina compared with control group.

### **2.5.2. Effects of Spirulina on body weight and body weight gain**

Mariey *et al.* (2012) reported that there was no significant on body weight of broilers fed dietary Spirulina which is similar to what was reported by Bonos *et al.* (2015) & Sugiharto *et al.* (2022). The effect of Spirulina supplementation in broiler shows that the live weight of the control birds and the birds supplemented with Spirulina did not differ significantly during the feeding experiment. Similarly, Zahir *et al.* (2019) reported that effect of Spirulina treatment on final live weight was not significant. In contrast, Fathi *et al.* (2018) reported that supplementing broiler chickens with *Spirulina platensis* significantly affected growth performance and live body weight at 38 days. Gongnet *et al.* (2001) reported that weight gains of birds fed diets with 100g/kg and 150g/kg of Spirulina had a significant reduction of 80 % and 70 % of the control diet. Pestana *et al.* (2020) reported that birds fed Spirulina diets supplemented with lysozyme had lower body weight in comparison to birds fed the control diet. Ross (1990) reported a growth depression with the diet containing 20% Spirulina as early as the 1<sup>st</sup> week. Chicks receiving 10% and 20% Spirulina grew significantly slower by the end of the 3<sup>rd</sup> week than those in control group. However, these results are not in agreement with that of Shanmugapriya *et al.* (2015) who reported that chicks supplemented with 1% Spirulina had the highest body weight gain (2162g) compared to control group (1847g). Ibrahim *et al.* (2018) also reported that, average body weight of supplemented groups with Spirulina was significantly higher compared to control group.

Gongnet *et al.* (2001) reported a decrease in weight gain to be associated with lysine deficiency in the formulated diet. However, Kharde *et al.* (2012) associated the differences in result to be due to some observed secondary parameters such as feed composition, housing conditions and production systems, causing the variation in the results. Table 2.3 shows the effect of supplementation of *Spirulina platensis* on feed intake, live body weight, body weight gain, growth performance and feed conversion ratio.

**Table 2.3** Effects of supplementation of *Spirulina platensis* to broiler diets on feed intake and growth performance

<b>Spirulina Diet (g)</b>	<b>Feed intake (g)</b>	<b>Live body weight (g)</b>	<b>Body weight gain (g)</b>	<b>Growth Rate (g/d)</b>	<b>Feed conversion ratio</b>	<b>Source</b>
<b>0</b>	3302.45	1711.72	1765.00	1.71	1.87	Fathi <i>et al.</i> , (2018)
<b>0.3</b>	3315.62	1966.45	1820.57	1.72	1.82	Fathi <i>et al.</i> , (2018)
<b>0.5</b>	3270.37	2014.60	1868.20	1.72	1.75	Fathi <i>et al.</i> , (2018)
<b>0.7</b>	3215.67	2016.80	1870.00	1.73	1.71	Fathi <i>et al.</i> , (2018)
<b>0.9</b>	3308.25	2078.25	1931.65	1.73	1.71	Fathi <i>et al.</i> , (2018)
<b>1</b>	1180.5	2729.3	574.6	-	2.06	Mirzaie <i>et al.</i> (2018)
<b>2</b>	1385.2	2769.4	602.2	-	2.3	Mirzaie <i>et al.</i> (2018)
<b>5</b>	-	2328	-	-	2.132	Bonos <i>et al.</i> , (2016)
<b>10</b>	-	2381	-	-	2.103	Bonos <i>et al.</i> , (2016)

### 2.5.3. Effects of *Spirulina* on feed conversion ratio (FCR)

Sugiharto *et al.* (2022) & Fathi *et al.* (2018) reported a no differences in FCR within the treatment groups when *Spirulina* was used as a supplement in broiler chicken diets. Zahir *et al.* (2019) supplemented broiler diets with *Spirulina* at 0, 0.5, 1 and 1.5%

inclusion levels and reported that the FCR was significantly low for birds fed diet supplemented with 0.5% (1.26) dried Spirulina powder than that of the control birds (1.45) but the overall mean FCR of different groups showed that there was no significant increase in groups fed with Spirulina compared to control groups. Kaoud (2012) also reported a lower feed conversion rate for birds supplemented with *Spirulina platensis* (1.78) compared with control birds (1.88). Gongnet *et al.* (2001) reported that a feed conversion for diets 0, 50, and 100 was within the range of good commercial production, whereas in diet 150, significantly more feed was necessary per unit body weight gain. Shanmugapriya *et al.* (2015) reported a lower FCR for birds supplemented with 1% Spirulina (1.716) than that of control birds (1.864). However, Pestana *et al.* (2020) reported that group fed Spirulina with or without enzyme supplementation had higher feed conversion ratios relative to the control group.

#### **2.5.4. Effects of Spirulina on poultry gut microflora**

Adil and Magray (2012) defined gut flora as organisms that exist in the digestive tract and are able to perform useful functions for their hosts. In addition, the digestive flora of poultry comprises of unicellular microorganisms particularly bacteria, fungi and protozoans. Most important of all, the growth of the gut microflora in birds includes numerous complex mechanisms and they are all dependent on several factors from the age of the birds to feed resources as well as the environment in which birds are raised (Yasothei, 2017). However, microbiota plays an important role in nutrition, cleaning of the body through removal of toxic compounds, growth performance and protection against pathogenic bacteria (Amit-Romach *et al.*, 2004).

Gut microflora directly interact with the lining of the gastrointestinal tract, and this can modify the physiology of the tract and immunological status of the bird (Van Leeuwen *et al.*, 2004). Gut microflora provides enzymes and substrates which together provides an extensive metabolome available for nutrient and energy derivation from the diet (Stanley *et al.*, 2013). To add to that, there is a correlation between the apparent metabolizable energy of the feed and microflora composition in the hindgut of the host; which is because of direct conversion of some dietary components into high energy metabolites by specialized bacteria. The use of such fermentation end products as lactic acid and short chain fatty acid would provide more energy for the host (Lan *et al.*, 2005) and improve the feed efficiency (Rinttilä & Apajalahti, 2013).

According to Ansari *et al.* (2018), supplementation of different levels of *Spirulina platensis* or prebiotic decreased coliforms count of ileum content, however, the count of ileal *Lactobacillus subtilis* increased in broilers fed with different levels of *Spirulina platensis* or prebiotic. Shanmugapriya *et al.* (2015) also got similar results in ileal digesta, where the *lactobacillus* counts increases in birds fed with 1 % of *Spirulina platensis*, whereas *E. coli* were significantly reduced compared to control group. Yusuf *et al.* (2016) recorded a non-significant change in the count of Staphylococci count in birds fed *Spirulina* in the two diet types when compare each dose with the control type. It was argued that, the supplementing *Spirulina* to a vegetarian protein diet in a dose of 2g/kg may reduce total bacterial and coliform counts of Japanese quails.

Contrary, Park *et al.* (2018) recorded no significant difference in coliform bacteria counts of broiler chickens fed with different levels of *Spirulina*. *Lactobacillus* counts were significantly increased linearly as dietary *Spirulina* supplementation increased.

Fathi *et al.* (2018) also got similar results of which they recorded a non-significant difference in total coliform between chicks fed diets supplemented with *Spirulina platensis* and the control group. Lactobacillus count was significantly increased in bird fed 0.9, 0.7 and 0.5 g of Spirulina compared with the control diet.

## **2.6.Importance of meat proximate composition**

Indigenous chickens have a prominent chicken flavor and texture. Consumer's interest in consumption of indigenous chickens has increased over the years. However, there is little scientific information explaining the flavor and the texture of the meat from these birds which is associated to the high demand of the meat. Moreover, there is few knowledge about the meat characteristics of indigenous chickens in traditional cuisines (Valavan *et al.*, 2016). The indigenous chicken's distinctive taste and texture when compared to that of broilers is yet to be assessed scientifically. Nutritionally, chicken meat is a source of protein (Marangoni *et al.*, 2015) and it is reported to have low energy which makes it one of the healthier food, and also due its reduced fat content (Table. 2.4) as well as higher polyunsaturated fatty acids when compared to other species meat (Riovanto *et al.*, 2012). Van Marle-Köster & Webb (2000) carried out an experiment on the carcass characteristics of South African native chickens lines, of which they recorded highest fat content (2.5%) in the Ovambo chicken; however they also observed the fastest growth rates in the Ovambo line. In addition to that, they recorded the lowest crude protein in the Cobb (14.12%).

**Table 2.4** Commercial cuts proximate composition (%) for four South African chicken breeds (Source: Packard, 2014)

<b>Parameter</b>	<b>Black Australorp</b>	<b>New Hampshire</b>	<b>Koekoek</b>	<b>Naked-Neck</b>
<b>Breast</b>				
Moisture	74.0	74.1	73.5	73.7
Crude protein	23.5	23.1	23.4	23.1
Lipid	2.1	2.2	2.51	2.4
Ash	1.1	1.1	1.1	1.1
<b>Thigh</b>				
Moisture	74.08	73.45	73.7	72.31
Crude protein	17.29	16.75	17.61	18.56
Lipid	7.6	8.5	7.2	8.0
Ash	1.0	1.0	1.0	1.1
<b>Drumstick</b>				
Moisture	75.7	75.2	75.0	73.6
Crude protein	19.6	19.1	19.5	19.7
Lipid	3.6	3.8	3.7	4.4
Ash	1.1	1.0	1.1	1.1

### **2.6.1. Effects of Spirulina on carcass yield and internal organs**

Carcass characteristics and cuts yield are essential attributes in determining portion yields for retail, this includes commercial cuts such as thigh, wing, drumstick, breast, neck and feet/shank. However, carcass characteristics parameters include parameters such as live slaughter weight, hot carcass weight, cold carcass weight and dressing percentage (Omojola *et al.*, 2004). Cold carcass weight is the weight of the carcass after 24 hours of cooling, while hot carcass weight is the carcass weight after slaughter

without the head, internal organs and intestines. Carcass characteristics are affected by many factors such as, amino acid content of the diet which support normal development in broiler chickens (Uushona, 2015). Furthermore, Omojola *et al.* (2004) reported that the dressing weight of broiler chickens is influenced by the weight of the visceral organs, age, slaughter weight, diet and sex. Singh & Pathak (2016) reported that hot carcass weight is affected by age and type of breed while cold carcass weight is affected by the stability of refrigeration storage which might be due to higher muscle activities in the chicken breed used.

Ansari *et al.* (2018) reported that different levels of Spirulina or prebiotic caused higher broiler carcass yield at day 42; however, breast (%), drumstick, thigh % and abdominal fat pad did not show significant differences among the treatment groups. Zahir *et al.* (2019) also recorded highest dressing percentage in Spirulina treated group although the effect was not significant. Moustafa *et al.* (2021) recorded a significant difference in dressing percentage, breast % and intestine % of chickens fed 1% Spirulina of which the highest yield was in also in chickens fed 1% Spirulina compared to 0.5% and 1.5% Spirulina. However, they recorded no significant difference in liver %, heart % and gizzard % among all treatments. Additionally, Mullenix *et al.* (2022) recorded a significant difference in breast and drumstick weights of which the highest weights for breast and drumstick was recorded in chickens fed control diet. A non-significant difference was recorded among the weights of wings and thighs.

Zahir *et al.* (2019) carried out a study on the effect of dietary supplementation of *Spirulina platensis* at different inclusion levels on growth performance of broiler chickens. They observed no significant difference in the relative weight of liver, gizzard,

intestine, heart and spleen (Table 2.5) of broiler chicks between the groups. Their results are similar to those of Kaoud (2012) who reported no significant difference in gizzard weight of broilers in all treatments when the experiment used Spirulina as a dietary supplement but observed a decrease in liver weight in Spirulina groups.

According to Akinmutimi *et al.* (2008) the release of trypsin inhibitors may cause the enlargement of internal organs such as pancreas and liver when animals are fed with legumes. Furthermore, liver enlargement may also be associated with increased metabolic activities of the liver in trying to make up for reduced availability of proteins that might have been made unavailable because of the presence of anti-nutritional factors (Okorie *et al.*, 2017). This is because the liver is a major cleansing organ and thus increase in its activities can result in possibly increased weight.

**Table 2.5** Effects of *Spirulina platensis* on internal organ weight (g) in broiler chicks (Source: Zahir *et al.*, 2019).

Parameters	<i>Spirulina platensis</i> inclusion levels			
	0%	0.5%	1%	1.5%
Liver (g)	35.83	37.89	37.33	35.56
Gizzard (g)	37.61	41.28	41.00	36.33
Intestine (g)	101.78	101.78	103.67	116.83
Heart (g)	6.72	7.39	6.11	7.44
Spleen (g)	1.44	1.94	1.73	1.78

### 2.6.2. Effects of Spirulina on meat quality

Meat quality is defined as a combination of factors differing from area to area and in context (Oliver *et al.*, 2006), hence, knowing the effects of feed ingredients on meat quality in poultry diets is crucial when determining what this feed could change on the

meat quality and eating quality and what this could mean for packers, retailers and consumers down the supply chain.

Researchers (Altmann *et al.*, 2018 and Park *et al.*, 2018; El-Bahr *et al.*, 2020; Pestana *et al.*, 2020 Moustafa *et al.*, 2021) found no significant difference in pH of the chicken breast muscle when Spirulina was used as an ingredient in the chicken diet. El-Bahr *et al.* (2020) furthermore recorded a no significant difference in thaw loss of breast muscle from chickens fed diet containing Spirulina and other microalgae species.

Altmann *et al.* (2018) determined the effects of feeding Spirulina and Hermetia as protein sources on meat quality of chickens. They reported that no significant change was recorded on Ph, cooking loss, storage loss and color when soybean meal was substituted with 50% Spirulina, while the inclusion of Spirulina at 50% resulted in a higher breast filet pH value at 24 h post mortem. Storage and cooking losses were reported to have decreased compared to the Hermetia meal and control group, and this may be attributed to the higher pH value. When pH increases significantly it lowers the water holding capacity (drip loss), resulting in a breast filet that is expected to be juicier (Aaslyng *et al.*, 2003) and tenderer in texture (Liu *et al.*, 2004).

## **2.7.Conclusion**

Spirulina platensis nutrients composition has demonstrated the potential it to be a good source of animal feed. Its high protein content has shown that Spirulina can be a good protein source. Furthermore, Spirulina is known for its good antioxidant properties which make it an excellent ingredient for poultry to promote gut health. Studies on the effect of Spirulina on growth performance has shown different results depending on the

inclusion level of Spirulina in the chicken`s diet. The high level of Spirulina inclusion in broiler diet has been reported by many sources to have caused detrimental effect on growth parameters and meat quality, while few have found it to have improved growth performance and others found no significant difference. Due to Spirulina`s prebiotic properties, it has been reported that Spirulina supplementation to poultry diets improve gut microflora by reducing coliforms and increasing *Lactobacillus*. It is therefore necessary to explore this alga which is a good source of protein and assess its effect on indigenous chickens or dual purpose as many literatures reviewed focused only on broilers and less attention was given to indigenous chickens which the rural communities are farming with.

## 2.8.References

- Aaslyng, M. D., Bejerholm, C., Ertbjerg, P., Bertram, H. C., & Andersen, H. J. (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food Quality and Preference*, 14: 277-288.
- Abdel-Daim, M. M. (2014). Pharmacodynamic interaction of *Spirulina platensis* with erythromycin in Egyptian Baladi bucks (*Capra hircus*). *Small Ruminant Research*, 120: 234-241.
- Adil, S., & Magray, S. N. (2012). Impact and manipulation of gut microflora in poultry: A review. *Journal of Animal and Veterinary Advances*, 11(6): 873–877.
- Agustini, T. W., Suzery, M., Sutrisnanto, D., & Ma'ruf, W. F. (2015). Comparative study of bioactive substances extracted from fresh and dried *Spirulina* sp. *Procedia Environmental Sciences*, 23: 282-289.
- Akinmutimi, A. H., Ojewola, G. S., Abasiokong, S. F., & Onwudike, O. C. (2008). Evaluation of toasted, cooked and akanwu-cooked sword bean meal in place of soya bean meal in broiler starter diets. *International Journal of Poultry Science*, 7 (5):480-486.
- Altmann, B. A., Neumann, C., Velten, S., Liebert, F., & Mörlein, D. (2018). Meat quality derived from high inclusion of a micro-alga or insect meal as an alternative protein source in poultry diets: A pilot study. *Foods*, 7: 34.
- Alvarenga, R. R., Zangeronimo, M. G., Pereira, L. J., Rodrigues, P. B., & Gomide, E. M.

- (2011). Lipoprotein metabolism in poultry. *World's Poultry Science Journal*, 67: 431-440.
- Anbarasan, V., Kumar, V. K., Kumar, P. S., & Venkatachalam, T. (2011). In vitro evaluation of antioxidant activity of blue green algae *Spirulina platensis*. *International Journal of Pharmaceutical Sciences and Research*, 2(10): 2616.
- Anonymous, (2022). Indigenous chicken breeds. <https://southafrica.co.za/indigenous-chicken-breeds.html> , retrieved 13 February 2022.
- Ansari, M. S, Hajati, H., Gholizadeh, F., Soltani, N.,& Alavi, M. (2018). Effect of different levels of spirulina platensis on growth performance, intestinal morphology, gut microflora, carcass characteristics and some blood parameters in broiler chickens. *Journal of Phycological Research*, 2(2): 187-197.
- Amit-Romach, E., Sklan, D., & Uni, Z. (2004). Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. *Poultry Science*, 83: 1093-1098.
- Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*. 25: 207-210.
- Bonos, E., Kasapidou, E., Kargopoulos, A., Karampampas, A., & Christaki, E. (2016). Spirulina as a functional ingredient in broiler chicken diets. *South African Journal of Animal Science*, 46(1): 95-102.
- Cavalier-Smith, T. (2007). Evolution and relationships. *Unravelling the Algae: The Past, Present, and Future of Algal Systematics; Brodie, J., Ed*, 21.

- Choe, J. H., Nam, K., Jung, S., Kim, B., Yun, H., & Jo, C. (2010). Differences in the quality characteristics between commercial Korean native chickens and broilers. *Korean Journal for Food Science of Animal Resources*, 30(1): 13–19.
- Chukwuebuka, E., & Chinenye, I. J. (2015). Biological functions and anti-nutritional effects of phytochemicals in living system. *Journal of Pharmarcy and Biological Science*, 10(2), 10-19.
- Cuc, N. T. K., Muchadeyi, F. C., Baulain, U., Eding, H., Weigend, S., & Wollny, C. B. A. (2006). An assessment of genetic diversity of Vietnamese H'mong chickens. *International Journal of Poultry Science*, 5(10): 905–913.
- Dykes, L., & Rooney, L. W. (2007). Phenolic compounds in cereal grains and their health benefits. *Cereal foods world*, 52(3): 105-111.
- El-Bahr, S., Shousha, S., Shehab, A., Khattab, W., Ahmed-Farid, O., Sabike, I., ... & Albosadah, K. (2020). Effect of dietary microalgae on growth performance, profiles of amino and fatty acids, antioxidant status, and meat quality of broiler chickens. *Animals*, 10(5): 761.
- El Din, S. M., Hussein, M. H., Hamouda, R. A., Shehawy, M. A., & Abd El Maksoud, A. I. (2019). Bioactive potentiality of some secondary metabolites extracted from microalga *Spirulina platensis*. *Journal of Chemical and Pharmaceutical Research*, 11(10): 22-35.
- Ennaji, H., Bourhia, M., Taouam, I., Falaq, A., Bellahcen, T. O., Salamatullah, A. M., & Cherki, M. (2021). Physicochemical evaluation of edible Cyanobacterium

- Arthrospira platensis collected from the south atlantic coast of Morocco: a promising source of dietary supplements. *Evidence-Based Complementary and Alternative Medicine*, 2021: 1-11.
- Fathi, M. A., Namra, M. M. M., Ragab, M. S., & Aly, M. M. M. (2018). Effect of dietary supplementation of algae meal ( *Spirulina platensis*) as growth promoter on performance of broiler chickens. *Egypt Poultry Science*, 38 (2): 375-389.
- Ferket, P.R., and Gernat, A.G. (2006). Factors that affect feed intake of meat birds: A review. *Intenational Journal of Poultry Science*, 5(10) :905-911.
- Gxasheka, M., Tyasi, T. L., Qin, N., & Lyu, Z. C. (2015). An overview of tannins rich plants as alternative supplementation on ruminant animals: a review. *International Journal of Agricultural Research and Review*, 3(6), 343-349.
- Gongnet, G. P., Niess, E., Rodehutsord, M., & Pfeffer, E. (2001). Algae-meal (*Spirulina platensis*) from lake Chad replacing soybean-meal in broiler diets. *Archiv Fur Geflugelkunde*, 65 (6): 265-268.
- Grobbelaar, J. A. N., Sutherland, B., & Molalako, N. M. (2010). Egg potentials of certain indigenous chicken breed from South Africa. *Animal Genetics Resources*, 46: 25-32.
- Hamouda, R. A., El-Boraey, N. G., El Bialy, B. E., Alrdahe, S. S., & Darwish, D. B. E. (2022). Vitamin supplements enhance *Spirulina platensis* biomass and phytochemical contents. *Green Processing and Synthesis*, 11(1), 266-274.
- Hassan, Z. M., Manyelo, T. G., Selaledi, L., & Mabelebele, M. (2020). The effects of

- tannins in monogastric animals with special reference to alternative feed ingredients. *Molecules*, 25(20): 4680.
- Hussein, R. A., & El-Anssary, A. A. (2019). Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal medicine*, 1(3).
- Ibrahim, S. S., Elsabagh, R., Allam, A., Youssef, G., Fadl, S. E., Abdelhiee, E. Y., ... & Aboubakr, M. (2021). Bioremediation role of *Spirulina platensis* against deltamethrin-mediated toxicity and its chemical residues in chicken meat. *Environmental Science and Pollution Research*, 28(40): 56188-56198.
- Kadam, S. U., Tiwari, B. K., & O'Donnell, C. P. (2013). Application of novel extraction technologies for bioactives from marine algae. *Journal of Agricultural and Food Chemistry*, 61: 4667-4675.
- Kingori, A. M., Tuitoek, J. K., Muiruri, H. K., Wachira, A. M., & Birech, E. K. (2007). Protein Intake of Growing Indigenous Chickens on Free-Range and Their Response to Supplementation. *International Journal of Poultry Science*, 6 (9): 617–621.
- Khalila, H. S., Fayed, W. M., Mansour, A. T., Srour, T. M., Omar, E. A., Darwish, S. I., & Moussa Nour, A. A. (2019). Dietary Supplementation of *Spirulina*, *Arthrospira platensis*, With Plant Protein Sources and their Effects on Growth, Feed Utilization and Histological Changes in Nile Tilapia, *Oreochromis niloticus*. *Journal of Aquaculture Research & Development*, 9: 549.
- Kaoud, H. A. (2012). Effect of *spirulina platensis* as a dietary supplement on broiler performance in comparison with prebiotics. *Scientific Journal of Applied Research*,

2 (2): 46–51.

Kharde, S. D., Shirbhate, R. N., Bahiram, K. B., & Nipane, S. F. (2012). Effect of Spirulina supplementation on growth performance of broilers. *Indian Journal of Veterinary Research*, 21(1): 66-69.

Kumar, K., Sinha, R. K., Kumar, S., Nirala, R. K., Kumari, S., & Sahu, S. P. (2022). Significance of tannins as an alternative to antibiotic growth promoters in poultry production. *The Pharma Innovation Journal*, 11: 1435-1440.

Lan, Y., Versteegen, M. W. A., Tamminga, S., & Williams, B. A. (2005). The role of the commensal gut microbial community in broiler chickens. *World's Poultry Science Journal*, 61(1): 95-104.

Liu, Y., Lyon, B. G., Windham, W. R., Lyon, C. E., & Savage, E. M. (2004). Principal component analysis of physical, color, and sensory characteristics of chicken breasts deboned at two, four, six, and twenty-four hours postmortem. *Poultry Science*, 83: 101-108.

Lo, D., Wang HsinI, W. H., Wu WanJen, W. W., & Yang RayYu, Y. R. (2018). Anti-nutrient components and their concentrations in edible parts in vegetable families. *CABI Reviews*, (2018), 1-30.

Manyelo, T.G.; Selaledi, L.; Hassan, Z.M.; Mabelebele, M. (2020). Local chicken breeds of Africa: Their description, uses and conservation methods. *Animals*, 10: 1-18.

Mahfuz, S., Shang, Q., & Piao, X. (2021). Phenolic compounds as natural feed additives

- in poultry and swine diets: A review. *Journal of Animal Science and Biotechnology*, 12(1): 1-18.
- Marangoni, F., Corsello, G., Cricelli, C., Ferrara, N., Ghiselli, A., Lucchin, L., & Poli, A. (2015). Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: An Italian consensus document. *Food and Nutrition Research*, 59: 1–11.
- Mariey, Y. A., Samak, H., & Ibrahim, M. (2012). Effect of using spirulina platensis algae as a feed additive for poultry diets: 1-productive and reproductive performances of local laying hens. *Egyptian Poultry Science*, 32 (1): 201-215.
- Matsuura, H. N., & Fett-Neto, A. G. (2015). Plant alkaloids: main features, toxicity, and mechanisms of action. *Plant toxins*, 2(7): 1-15.
- Menge, E. O., Kosgey, I. S., & Kahi, A. K. (2005). Bio-economic model to support breeding of indigenous chicken in different production systems. *International Journal of Poultry Science*, 4(11):827-839.
- Mirzaie, S., Zirak-khattab, F., Hosseini, S. A., & Donyaei-darian, H. (2018). Effects of dietary Spirulina on antioxidant status , lipid profile , immune response and performance characteristics of broiler chickens reared under high ambient temperature. *Asian-Australasian Journal of Animal Sciences*, 31(4): 556–563.
- Motsepe, R., Mabelebele, M., Norris, D., Brown, D., Ngambi, J., & Ginindza, M. (2016). Carcass and quality characteristics of sSouth African indigenous chickens. *Indian Journal of Animal Research*, 50: 580-587.

- Moustafa, E.S.; Alsanie, W.F.; Gaber, A.; Kamel, N.N.; Alaqil, A.A.; Abbas, A.O. (2021). Blue-Green Algae (*Spirulina platensis*) Alleviates the Negative Impact of Heat Stress on Broiler Production Performance and Redox Status. *Animals*, 11 (1243):1-13.
- Mullenix, G. T., Maynard, C. J., Owens, C. M., Rochell, S. J., Bottje, W. G., Brister, R. D., & Kidd, M. T. (2022). *Spirulina platensis* meal inclusion effects on broilers fed a reduced protein diet. *Journal of Applied Poultry Research*, 31: 100199.
- Myers, N., & Kent, J. (2003). New consumers: The influence of affluence on the environment. *Proceedings of the National Academy of Sciences*, 100 (8): 4963-4968.
- National Research Council (NRC). (1994). Nutrient requirements of poultry 9<sup>th</sup> revised ed.. National Academy of Sciences. Washington, DC, USA.
- Nasr, J., Kheiri, F., Solati, A., Hajibabaei, A., & Senemari, M. (2011). The efficiency of energy and protein of broiler chickens fed on diets with different lysine concentrations. *Journal of Animal and Veterinary Advances*, 10: 2394–2397.
- Nasr, J., & Kheiri, F. (2012). Effects of lysine levels of diets formulated based on total or digestible amino acids on broiler carcass composition. *Brazilian Journal of Poultry Science*, 14: 249-258.
- Okorie, K. C., Esiegwu, A. C., & Okonkwo, U. N. (2017). Evaluation of the growth performance, carcass characteristics and blood indices of broiler finishers fed graded levels of fermented *Mucuna sloanei* seed meal. *Journal of Tropical*

*Agriculture, Food, Environment and Extension*, 15: 39-44.

Oliver, M. A., Nute, G. R., Font i Furnols, M., San Julián, R., Campo, M. M., Sañudo, C., ... Montossi, F. (2006). Eating quality of beef, from different production systems, assessed by German, Spanish and British consumers. *Meat Science*, 74: 435-442.

Omojola, A. B., Adeshinwa, A. O. K., Madu, H., & Attah, S. (2004). Effect of sex and slaughter weight on broiler chicken carcass. *Journal of Food, Agriculture & Environment*, 2:61-63.

Packard, R. (2014). Comparison of Production Parameters and Meat Quality Characteristics of South African Indigenous Chickens. <http://hdl.handle.net/10019.1/86211> ,Retrieved from <http://scholar.sun.ac.za> 02/07/2022.

Park, J. H., Lee, S. I., & Kim, I. H. (2018). Effect of dietary *Spirulina* (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. *Poultry science*, 97(7): 2451-2459.

Pestana, J.M., Puerta, B., Santos, H., Madeira, M. S., Lopes, P. A., Pinto, R. M. A., Lemos, J. P. C., Fontes, C. M. C. A., Lordelo, M. M., & Prates, J. A. M. (2020). Impact of dietary incorporation of *Spirulina* (*Arthrospira platensis*) and exogenous enzyme on broiler performance, carcass traits and meat quality. *Poultry Science*, 99 (5): 1-14.

- Petrus, N.P., Kangootui, K., Kandiwa, E., Madzingira, O., & Mushonga, B. (2019). Relationship of age and live weight to linear body traits in female intensively reared Boschvel chickens in Namibia. *International Journal of Poultry Science*, 18 (10):1-9.
- Plaza, M., Herrero, M., Alejandro Cifuentes, A., & Ibáñez, E. (2009). Innovative natural functional ingredients from microalgae. *Journal of Agricultural and Food Chemistry*, 57: 7159- 7170.
- Prinajati, P. D. (2021). Analysis of reducing co2 emissions using Spirulina microalgae. *Journal of Community Based Environmental Engineering and Management*, 5(1), 47-56.
- Ramteke, R., Doneria, R., & Gendley, M. K. (2019). Antinutritional factors in feed and fodder used for livestock and poultry feeding. *Acta scientific nutritional Health*, 3(5): 39-48.
- Rajauria, G., Cornish, L., Ometto, F., Msuya, F. E., & Villa, R. (2015). Identification and selection of algae for food, feed, and fuel applications. *Seaweed Sustainability*, (pp. 315-345). Academic Press.
- Rashwan, R. S., & Hammad, D. M. (2020). Toxic effect of Spirulina platensis and Sargassum vulgar as natural pesticides on survival and biological characteristics of cotton leaf worm Spodoptera littoralis. *Scientific African*, 8, e00323.
- Ravindran, V. (2013). Feed enzymes: The science, practice, and metabolic realities. *Journal of Applied Poultry Research*, 22(3): 628–636.

- Rinttilä, T., & Apajalahti, J. (2013). Intestinal microbiota and metabolites-Implications for broiler chicken health and performance. *Journal of Applied Poultry Research*, 22: 647-658.
- Riovanto, R., De Marchi, M., Cassandro, M., & Penasa, M. (2012). Use of near infrared transmittance spectroscopy to predict fatty acid composition of chicken meat. *Food Chemistry*, 134: 2459-2464.
- Ross, E., & Dominy, W. (1990). The nutritional value of dehydrated, blue-green algae (*Spirulina plantensis*) for poultry. *Poultry science*, 69(5): 794-800.
- Saleh, A. A., Amber, K. A., Soliman, M. M., Soliman, M. Y., Morsy, W. A., Shukry, M., & Alzawqari, M. H. (2021). Effect of low protein diets with amino acids supplementation on growth performance, carcass traits, blood parameters and muscle amino acids profile in broiler chickens under high ambient temperature. *Agriculture*, 11(2): 185.
- Sarker, P. K., Gamble, M. M., Kelson, S., & Kapuscinski, A. R. (2016). Nile tilapia (*Oreochromis niloticus*) show high digestibility of lipid and fatty acids from marine *Schizochytrium* sp. and of protein and essential amino acids from freshwater *Spirulina* sp. feed ingredients. *Aquaculture Nutrition*, 22(1): 109–119.
- Saranraj, P., & Sivasakthi, S. (2014). *Spirulina Platensis* – Food for Future : A Review. *Asian Journal of Pharmaceutical Science & Technology*, 4(1): 26-33.
- Selaledi, L., Baloyi, J., Mbajjorgu, C., Sebola, A. N., Kock, H. D., & Mabelebele, M. (2021). Meat Quality Parameters of Boschveld Indigenous Chickens as Influenced

by Dietary Yellow Mealworm Meal. *Foods*, 10(12): 3094.

Shanmugapriya, B., Babu, S. S., Hariharan, T., Sivaneswaran, S., Anusha, M. B., & College, C. N. (2015). Dietary administration of spirulina platensis as probiotics on growth performance and histopathology in broiler chicks. *International Journal of Recent Scientific Research*, 6(2): 2650-2653.

Shiningavamwe, K. L. (2022). *Nutrient content and the effects of feeding four Namibian encroacher bush species on growth, methane production and carcass characteristics of Damara sheep* (Doctoral dissertation, University of Namibia).

Singh, V.P., & Pathak, V. (2016). Contribution of carcass cuts in meat production of Kadaknath, Aseel and Vanraja breeds of chicken. *Journal of Animal Research*, 7(1):213-217.

Stanley, D., Geier, M. S., Denman, S. E., Haring, V. R., Crowley, T. M., Hughes, R. J., & Moore, R. J. (2013). Identification of chicken intestinal microbiota correlated with the efficiency of energy extraction from feed. *Veterinary Microbiology*, 164(1–2): 85–92.

Sugiharto S, Atmaja BM, Widiastuti E, Hadiyanto H. (2022). Combined use of *Spirulina platensis* and *Saccharomyces cerevisiae*: Implication on growth, blood profile and intestinal morphology and bacteria of the Indonesian crossbred chickens. *Biodiversitas*, 23: 160-165.

Suleyman, C. (2017, May). Tannins and their effects on poultry nutrition. In *International Conference Agriculture, forestry, food Science and Technology*.

*Cappadocia, Turkey*, (pp. 15-17).

Tadelle, D., & Fasil, G. (2016). The Potchefstroom Koekoek Breed. *African Chicken Genetic Gains. The International Livestock Research Institute: Nairobi, Kenya.*  
<https://core.ac.uk/download/pdf/132683028.pdf> retrieved 21 February 2022.

Teferedegne, B. (2000). New perspectives on the use of tropical plants to improve ruminant nutrition. *Proceedings of the Nutrition Society*: 59(2): 209-214.

Thormar, H. (2012). Patented Non-Antibiotic Agents as Animal Feed Additives. *Recent Patents on Food, Nutrition & Agriculture*, 4:1-14.

Uushona, T. (2015). Black soldier fly (*Hermetia illucens*) pre-pupae as a protein source for broiler production. M.Sc. Thesis. University of Stellenbosch, South Africa.

Yacout, M. H. M. (2016). Anti-nutritional factors & its roles in animal nutrition. *Journal of Dairy Veterinary and Animal Research*, 4(1): 237-239.

Van Krimpen, M. M. , Bikker, P., Van der Meer I. M., Van der Peet-Schwering , C.M.C., & Vereijeke, J.M. (2013). Cultivation, processing and nutritional aspects for pigs and poultry of European protein sources as alternatives for imported soybean products. *Wageningen UR, livestock report*, 662 (February).

Van Marle-Köster, E., & Webb, E. C. (2000). Carcass characteristics of South African native chicken lines. *South African Journal of Animal Sciences*, 30(1): 53-56.

Van Marle-Köster, E. (2001). Genetic and phenotypic characterization of native fowl

- populations in South Africa. Doctoral dissertation, University of Pretoria, South Africa.
- Van Leeuwen, P., Mouwen, J. M. V. M., Van Der Klis, J. D., & Verstegen, M. W. A. (2004). Morphology of the small intestinal mucosal surface of broilers in relation to age, diet formulation, small intestinal microflora and performance. *British Poultry Science*, 45(1): 41-48.
- Valavan, S. E., Omprakash, A.V., Bharatidhasan, A., & Kumar, V. R. S. (2016). Comparison of nutrient composition of native chicken and commercial broiler under Indian condition. *International Journal of Applied and Pure Science and Agriculture*, 2 (12):7-11.
- Vonshak, Avigad, Cheung, S. M., & Chen, F. (2000). Mixotrophic growth modifies the response of *Spirulina* (*Arthrospira*) *platensis* (Cyanobacteria) cells to light. *Journal of Phycology*, 36 (4): 675-679.
- Wang, H., Zhao, X., Lin, M., Randy A, D., Chen, W., Zhou, J., & Bao, Q. (2013). Proteomic analysis and qRT-PCR verification of temperature response to *Arthrospira* (*Spirulina*) *platensis*. *Proteome Science*, 11:6.
- Yasothai, R. (2017). Importance of gut microflora in poultry. *International Journal of Science, Environment and Technology*, 6(1): 549–552.
- Younas, M., & Yaqoob, M. (2005). Feed resources of livestock in the Punjab, Pakistan. *Livestock research for rural development*, 17(2):18 Retrieved August 19, 2022, from <http://www.lrrd.org/lrrd17/2/youn17018.htm>

Yusuf, M. S., Hassan, M. A., Abdel-daim, M. M., Nabtiti, A. S. El, & Ahmed, A. M. (2016). Value added by *Spirulina platensis* in two different diets on growth performance , gut microbiota , and meat quality of Japanese quails. *Veterinary World*, 9 (11):1287–1293.

Zahir, U. R., Md. Anwarul, H. B., Maksuda, B., & Noushin, A. (2019). Effects of dietary supplement of dried *Chlorella* powder as an alternative to antibiotic on growth performance and health status of broiler chicken. *International Journal of Biosciences*, 14 (4): 255-263

## CHAPTER 3

### EFFECTS OF FEEDING INCREMENTAL LEVELS OF *SPIRULINA PLATENSIS* ON FEED INTAKE AND GROWTH PERFORMANCE OF BOSCHVELD AND POTCHEFSTROOM KOEKOEK CHICKEN BREEDS

#### Abstract

Spirulina has become a trend in the feed manufacturing industry as it is considered to be rich in essential nutrients especially proteins for humans and animals. The present study was conducted to determine the effect of substituting soybean meal with incremental levels of Spirulina on feed intake and growth performance of Boschveld and Potchefstroom Koekoek chicken breeds. A 16 weeks feeding trial was conducted using 72 unsexed chickens from the two chicken breeds, divided equally among the breeds. The birds were fed with commercial starter crumbles from day 1 to week 4. At week 5 of age, the chickens were randomly allocated to the four experimental diets which were as follows; 0% SP, 5% SP, 10% SP and 15% SP of dried *Spirulina platensis* powder as a replacement of soybean meal. The birds were fed the grower experimental diets for 12 weeks. Feed intake data were collected daily and live weights were taken weekly for growth performance data. The results showed that feed intake responded negatively to increase in Spirulina inclusion level. Boschveld (BV) chickens had high feed intake ( $P>0.05$ ) than Potchefstroom Koekoek (PK). However, for all diets, there was an increase in feed intake with age of birds. Chickens on diet 0% SP had higher weights ( $P<0.05$ ) which was not affected ( $P>0.05$ ) by the breed. Breed had a significant effect

( $P < 0.05$ ) on ADG where the BV had higher mean values (1.64g) compared to PK (1.57g). The feed conversion ratio (FCR) was highest ( $P < 0.05$ ) in chickens on diet 10% SP, with a mean value of 1.14 and the lowest in 0% SP with the value of 0.93. Birds on diet 0% SP recorded the highest PER (0.91) while those on diet 15% SP had the least PER (0.57). The highest PER value was recorded in week 2 of 1.22. In conclusion, replacing soybean meal with incremental levels of *Spirulina* negatively affected feed intake, body weights and PER. FCR was increased with increasing level of *Spirulina* in the diet. About 5% of *Spirulina* can be beneficial in terms of CP intake. However, increasing *Spirulina* level in the chicken diet above 5 % decreases feed intake, PER and average weights of the birds. *Spirulina* have the potential to be used as a protein source supplement in indigenous chicken feeds due to its high level of protein.

**Key words:** Breed, FCR, live weight, growth performance

### **3.1. Introduction**

Indigenous chickens are linked with rural households and they play important economic and social roles to the rural communities (Packard, 2014). The increase in human population together with urbanization and increasing incomes have resulted to a surge in demand for animal protein. Many emerging farmers in Namibia are venturing into poultry production, mainly indigenous chicken farming but feeds remain a big challenge. Soybean is the leading conventional protein source for animal feeding, but the current allocations of soybean is unsustainable due to the climate that is unsuitable for soya operations (Bagopi *et al.*, 2016). In addition, due to the competition of food between humans and animals especially monogastric, the cost of feeding chickens for maximum growth performance has increase, this has resulted in a very little contribution of indigenous chickens to food security as production of indigenous chickens on a larger scale is far from materializing (Sebola, 2015). This is primarily related to their relatively low productive performances caused by their slow growth (Magothe *et al.*, 2012).

Many households in rural areas raise indigenous chickens due to their adaptability (Masaire *et al.*, 2018) and low feed consumption compared to exotic breeds (Van Marle-Koster & Webb, 2000). The nutritional requirements of indigenous chickens differ from that of exotic strains, with the exotic strains requiring feed of high nutritional value which is mostly also used as man food (Sebola, 2015). This also contributed to indigenous chickens being preferred in rural areas as their production can be operated on a subsistence production system where they can be kept as scavengers with low cost

(Akinola & Essien, 2011). Rural farmers depend on the hens to perform hatching and taking care of the chicks which ends in long production cycles as they take longer to reach maturity and slaughter weight (Petrus, 2011). For indigenous chickens to promote efficiently food security, it is important to advance and promote production of local chickens (Sebola, 2015). Therefore, it is important to identify new feedstuffs that are of high importance to sustain animal production and future production of indigenous chickens. The new feedstuffs are required to have high nutritional value and be able to be utilized efficiently by the animal, able to utilize land and water efficiently and overall should result in an animal product that is of good quality and acceptable by consumers (Poppi & McLennan, 2010).

Natural environments could be a significant biological source of useful ingredients for example exploring algae (i.e. *Spirulina platensis*) as alternative source of protein (Lordan *et al.*, 2011; Draaisma *et al.*, 2013). The utilization of microalgae as feed ingredient can contribute to the safety of the environment and natural resources, specifically land degradation and water deprivation (Pestana *et al.*, 2020). *Spirulina platensis* is blue-green algae, edible, high in protein and able to survive in harsh environment and has gained popularity in exploration as food for both man and animals. Dietary Spirulina has been associated with increased growth performance and feed conversion of chickens (Park *et al.*, 2018; Zahir *et al.*, 2019). Limited researches were done on how to improve the output of indigenous chickens under intensive systems and using Spirulina as a protein source. Therefore, the objective of this study was to determine the effect of dietary inclusion of *Spirulina platensis* as a replacement of soybean meal on the feed intake and growth performance of Potchefstroom Koekoek and Boschveld chickens.

## **3.2. Materials and Methods**

### **3.2.1. Bird management and care**

The study was conducted at the University of Namibia, Neudamm campus, which is situated approximately 35km east of, Windhoek. A total of 72 one-day old unsexed indigenous chicks consisting of 36 Boschveld (BV) and 36 Potchefstroom Koekoek (PK) were sourced from a local indigenous poultry producer in Windhoek. The chicks were vaccinated against Gumboro, Infectious Bronchitis (IB) and Newcastle disease at day one. About one week before the arrival of chicks, all facilities and equipment including the brooding area were thoroughly cleaned and disinfected with veterinary disinfectant F10 (Health and Hygiene (Pty) LTD, South Africa) to eliminate the disease-causing organisms.

The brooder house was pre-heated with infra-red bulbs to keep it warm. The day-old chicks were kept in the brooder for four weeks. Bulbs were turned on during the night to encourage eating and to keep the chicks warm. Newspapers beddings were used as insulator throughout the brooding phase. During the brooding period feeds were placed on the feeding trays for chicks. Feeding troughs were used after the brooding period. Waterers were washed daily before being filled with water. The litter materials were replaced every second day during brooding and subsequently every week, until the end of the experiment. For the first four weeks, chicks were fed with commercial broiler starter diet alone. Good animal practice was observed during the entire duration of the study. The university veterinarians on campus were contacted for required animal care such as, treatment, proper sanitation and observations of birds when there was a concern

and on advice about the coccidiostat and products to use in cleaning the drinkers to be used for the bird`s water.

### **3.2.2. Experimental design**

The experiment was a 2 x 4 factorial arrangement in a complete randomized design (CRD) with factor one as two chicken breeds of Boschveld and Potchefstroom Koekoek and factor two as the four dietary treatments with different levels of *Spirulina platensis* inclusion as replacement of soybean meal (0% SP; 5% SP; 10% SP and 15%SP). From week 5 within each breed, three birds were randomly assigned to each of three cages (of dimension 0.9 m x 0.6 m x 0.6 m) with a single dietary treatment. Hence the experimental unit was a cage holding three birds and there were three replicates within each breed-diet treatment combination, giving a total of 72 birds. Excluding the brooding period (1-4 weeks), the experiment during the growth phase lasted for 12 weeks.

### **3.2.3. Treatment diets formulation**

The *Spirulina platensis* used in this experiment was sourced from Germany (Brandenburg University of Technology). The treatment diets (grower diets) formulated with graded levels of dried *Spirulina platensis* powder as replacement for soybean meal per 100kg of feed were: 0% SP, 5%SP, 10%SP and 15%SP. The four final diets were fed as mash and they were offered to the birds during the grower phase (5-16 weeks). The ingredients used to formulate the grower experimental diets are shown in Table 3.1.

**Table 3.1** Ingredients used for the experimental diets during the growth-phase (5-16 weeks)

Ingredient (%)	Experimental diets			
	0% SP	5% SP	10% SP	15% SP
Yellow maize	59	59	59	59
Wheat bran	20.8	20.8	20.8	20.8
Soybean meal	19.5	14.5	9.5	4.5
<i>Spirulina platensis</i>	0	5	10	15
Vitamin& Mineral premix	0.2	0.2	0.2	0.2
Common salt (NaCl)	0.2	0.2	0.2	0.2
Sunflower oil	0.3	0.3	0.3	0.3
Total	100	100	100	100

#### 3.2.4. Chemical composition analysis of the experimental diets

The samples of the *Spirulina platensis*, soybean meal and of the four diets were dried and milled through a 1mm screen before laboratory analyses (Table 3.2). The dry matter (DM) content of feeds was determined by drying the samples in oven at 105 °C for 24 h (AOAC, 2016). Ash was determined by incineration in a muffle furnace at 550 °C for 6 h (AOAC, 2016). The AOAC (2016) method no. 978.04 was used to determine the total nitrogen content of the samples and the CP was determined using a Leco protein

analyzer calibrated with EDTA (Leco Corporation) estimated by multiplying percentage of nitrogen (N) content by a factor 6.25. Ether extract (EE) was determined using the AOAC method 920.39 (AOAC, 2016). The procedure described by Van Soest *et al.* (1991) was used to determine neutral detergent fiber (NDF) and acid detergent fiber (ADF) using the Ankom 200 fiber analyzer.

The concentration of macro and micro elements was analysed using the inductively coupled plasma-atomic emission spectrometry (ICP-AES) (icap 6000 series) methods (AOAC, 2016), except for phosphorus (P) content which was determined using UV-vis-spectrophotometry (AOAC, 2016).

### **3.2.5. Anti-nutritional factors: Total phenols and Tannins content by Folin Ciocalteu method**

The determination of total phenols (TP), total non-tannins (NTP), total tannins (TT), condensed tannins (CT) and hydrolysable tannins (HT) was done according to a modified method of Makkar (2003). For TP, a 200mg of well ground samples were weighed in 15ml centrifuge tubes and dissolved in 10ml of 70% acetone. The samples were kept in ultrasonic water bath (Bransonic ultrasonic cleaner, 8510E-DHT) at 25°C for 35 minutes. After the samples were removed from the water bath, cooled down in a fridge which was set at 4°C for 5 minutes and centrifuged at 3000 rpm for 10 minutes in a Hermle Labortechnik GmbH Z366 centrifuge. The original extract was collected in a separate 15ml centrifuge tube and kept refrigerated for analysis. Polyvinyl-polyrrolidone (PVPP) was used to separate tannin phenols from NTP. The TP and NTP were both calibrated against tannic acid as a standard, the TT was calculated as a

difference between TP and NTP and all values were expressed as tannic acid equivalents. Absorbances were recorded at 725nm using a spectrophotometer (Spectro UV-11). For CT, a sample extract of 250µl was put in a centrifuge tube followed by 1500µl of Butanol-HCL reagent and 50µl of ferric reagent. The centrifuge tubes were covered and heated in a water bath at 98°C for 1 hour. The tubes were then cooled down and room temperature and absorbance read at 550nm using a spectrophotometer (Spectro UV-11). The CT content was expressed as leucocyanidin equivalent. The difference between TT and CT was used to calculate for HT (Table 3.3).

**Table 3.2** Chemical composition and mineral content of the experimental diets, Soybean meal and *Spirulina platensis* g/kg DM

Parameters	Diets					
	<i>Spirulina platensis</i>	Soybean meal	0% SP	5% SP	10% SP	15% SP
Dry matter (g/kg DM)	928.7	934.50	905.75	908.20	910.50	909.55
Ash (g/kg DM)	771.50	60.35	41.90	42.15	41.60	44.50
Ether extract	2.80	23.25	71.70	68.80	73.35	73.10
Crude protein	712.00	499.00	181.00	207.00	204.50	216.00
NDF	85.50	121.35	410.25	461.10	463.55	448.65
ADF	143.00	58.20	53.90	62.35	67.05	56.05
Metabolizable Energy (Kcal/kg)	3467.97	3704.59	3959.13	3945.98	3979.45	3971.08
K	12.40	18.65	91.00	9.35	8.65	8.40
Mg	2.20	2.20	2.35	2.15	2.30	2.25
Na	1.00	0.04	0.97	0.99	1.82	2.36
Ca	0.70	2.31	1.00	0.96	0.88	0.82
Cu	0.01	0.01	0.03	0.02	0.02	0.02
Fe	0.44	0.10	0.14	0.14	0.17	0.24
Mn	0.02	0.04	0.12	0.11	0.12	0.13
Zn	0.02	0.05	0.14	0.11	0.10	0.12

**Table 3.3** The concentration of phenols and tannins (g/kg DM) in *Spirulina platensis*, soybean meal and experimental diets

	<b>TP</b>	<b>NTP</b>	<b>TT</b>	<b>CT</b>	<b>HT</b>
SB	30.5	7.75	22.75	5.78	16.97
SP	74.2	19.4	54.8	10.07	44.73
0%SP	32.4	7.3	25.1	4.28	20.82
5%SP	59.2	7.7	51.5	6.94	44.56
10%SP	59.15	10.85	48.3	7.01	41.29
15%SP	60.85	15.2	45.65	7.14	38.51

SB: soybean meal; SP: *Spirulina platensis*; TP: total phenols; NTP: non-total phenols; CT: condensed tannins; HT: hydrolysable tannins

### 3.2.6. Production parameters measurements

Feed and water were provided *ad libitum* during the 16-week experimental period. For the purpose of this document, the results to be reported are from week 5-16, of which week 5 will be referred to as week 1 and week 16 will be referred to as week 12 during the data collection period. Average daily feed intake per bird was measured from week 1 to week 12 weeks of age by subtracting the weight of the feed refusals from that of the feed offered per day and dividing the difference by the total number of birds in a cage.

The weight of individual birds at week four before they were allocated to their experimental diets was used as initial weight in this experiment. Individual body weights of the birds in a cage were measured weekly until slaughter. The data for daily feed intake and live body weight were used to calculate the weekly feed intake, feed conversion efficiency (FCR), average daily gain (ADG), crude protein intake (PI) and protein efficiency ratio (PER). The PI (equation 3.2) were calculated according to

Despins & Axtell (1995) and the PER (equation 3.4) were calculated according to Boling-Frankenbach *et al.* (2001). The number of chickens that died during the experiment was recorded on a daily basis.

The following formulae were used for different variables (Equation 3.1-3.4):

**Equation 3.1**

$$\text{Average daily gain} = \frac{(\text{Average weight per chicken at week 16} - \text{Average weight per chicken at week 5})}{\text{Age (days)}}$$

**Equation 3.2**

$$\text{Crude protein intake} = \text{Feed intake} \times \text{Crude protein \%}$$

**Equation 3.3**

$$\text{Feed conversion ratio} = \frac{\text{Cumulative feed intake (g)}}{\text{Average weight of chicken (g)}}$$

**Equation 3.4**

$$\text{Protein efficiency ratio} = \frac{\text{Body weight change}}{\text{Crude protein intake}}$$

**3.2.7. Statistical analyses**

The data on daily feed intake, CP intake, body weight, protein efficiency ratio and feed conversion efficiency were analyzed using Proc Mixed procedure of SAS (2009), where the initial weight was used as a covariate. To account for the correlation of measurements on an experimental unit the best covariance structure was selected using

the Bayesian Information Criterion (BIC) (Littell *et al.*, 1998). The best covariance structures were: compound symmetry heterogonous (CSH) for feed intake; Huynh-Feldt (HF) for CP intake; variance components (VC) for body weight; compound symmetry (CS) for FCR; and Heterogeneous Autoregressive (AR [1]) for PER. The following linear model (1) was used for analysis:

$$Y_{ijkl} = \mu + D_i + B_j + W_k + (D \times B)_{ij} + (B \times W)_{jk} + (D \times W)_{ik} + (D \times B \times W)_{ijk} + E_{ijkl}$$

Where  $Y_{ijk}$  is the observation of the treatment of the dependent variable  $ijk$ ,  $\mu$  the fixed effect of population mean variable,  $D_i$  = the effect of  $i^{\text{th}}$  treatment diet (0% SP, 5% SP, 10% SP, 15% SP);  $B_j$  = the effect of  $j^{\text{th}}$  chicken breed (BV, PK);  $W_k$  = the effect of the  $K^{\text{th}}$  week ( $k = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12$ );  $(D \times B)_{ij}$  = the interaction effect of the  $i^{\text{th}}$  diet and  $j^{\text{th}}$  chicken breed;  $(B \times W)_{jk}$  = the interaction effect between  $j^{\text{th}}$  chicken breed and the  $k^{\text{th}}$  week;  $(D \times W)_{ik}$  = the interaction effect between the  $i^{\text{th}}$  treatment diet and the  $k^{\text{th}}$  week;  $(D \times B \times W)_{ijk}$  = the interaction effect between the  $i^{\text{th}}$  diet,  $j^{\text{th}}$  chicken breed and the  $k^{\text{th}}$  week;  $E_{ijkl}$  = the random error. Significance level was set at  $P < 0.05$ . Means indicating significant differences were tested using Duncan`s multiple range test.

### 3.3. Results

#### 3.3.1 Feed intake

The statistical significance (p values) of the effect of the main factors (diet, chicken breed and weeks) and their interaction on feed intake and protein intake are given in Table 3.4.

**Table 3.4** Statistical significance of treatment diets, breed and week, and their interactions on the feed intake parameters as influenced by incremental levels of Spirulina in the diet.

Parameters	Effect of treatment			Interaction			
	Diet	Breed	Week	DxB	DxW	BxW	DxBxW
FI	*	NS	***	NS	*	***	NS
PI	NS	NS	***	**	***	***	NS

D= Treatment Diet; B= Chicken breed; W=Week; FI= Feed intake; PI= Protein intake; \*P<0.05; \*\*P<0.001; \*\*\*P<0.0001; NS= not significant

The results of the study show that 3-way interactions (breed x diet x weeks) did not (P>0.05) influence feed intake as shown in Table 3.4. Similarly, the 2-way interactions of diet x breed did not (P>0.05) affect feed intake as shown in Table 3.5. However, the dietary treatment significantly (P<0.05) influenced feed intake. Chickens offered 0% SP had higher (P<0.05) daily feed intake, 65.33g than chickens on diets 5% SP, 10% SP and 15% SP as shown in Table 3.6. Among the Spirulina diets, chickens on 5% SP had

greater ( $P<0.05$ ) feed intake of 57.56g compared to 10% SP and 15% SP which did not differ ( $P>0.05$ ) and had mean values of 53.60g and 52.88g, respectively, as shown in Table 3.6. Furthermore, there were significant ( $P<0.05$ ) differences in feed intake among the measurement weeks of the study (Figure 3.1). For all the treatment diets, there was an increase in feed intake with time (weeks) as the chickens increase in sizes. The lowest feed intake ( $P<0.05$ ) was recorded in week 1 (40.54g/day) and the highest was recorded in week 11 and 12 of 64.11g/day and 64.65g/day, respectively.

**Table 3.5** Effects of diet and breed interactions on feed intake parameters of Boschveld and Potchefstroom Koekoek chickens.

<b>Breed</b>	<b>Diet</b>	<b>FI (g)</b>	<b>PI (g)</b>
BV	0% SP	66.36	11.97 <sup>a</sup>
	5% SP	58.92	12.14 <sup>a</sup>
	10% SP	53.71	11.16 <sup>b</sup>
	15% SP	54.67	11.90 <sup>a</sup>
PK	0% SP	64.31	11.62 <sup>a</sup>
	5% SP	56.20	11.69 <sup>a</sup>
	10% SP	53.49	10.79 <sup>b</sup>
	15% SP	51.09	10.96 <sup>b</sup>
<b>SEM</b>		2.5701	0.5193
<b>P-value</b>		0.2603	0.0010

<sup>ab</sup>Means with different superscripts within a column differ ( $P<0.05$ )

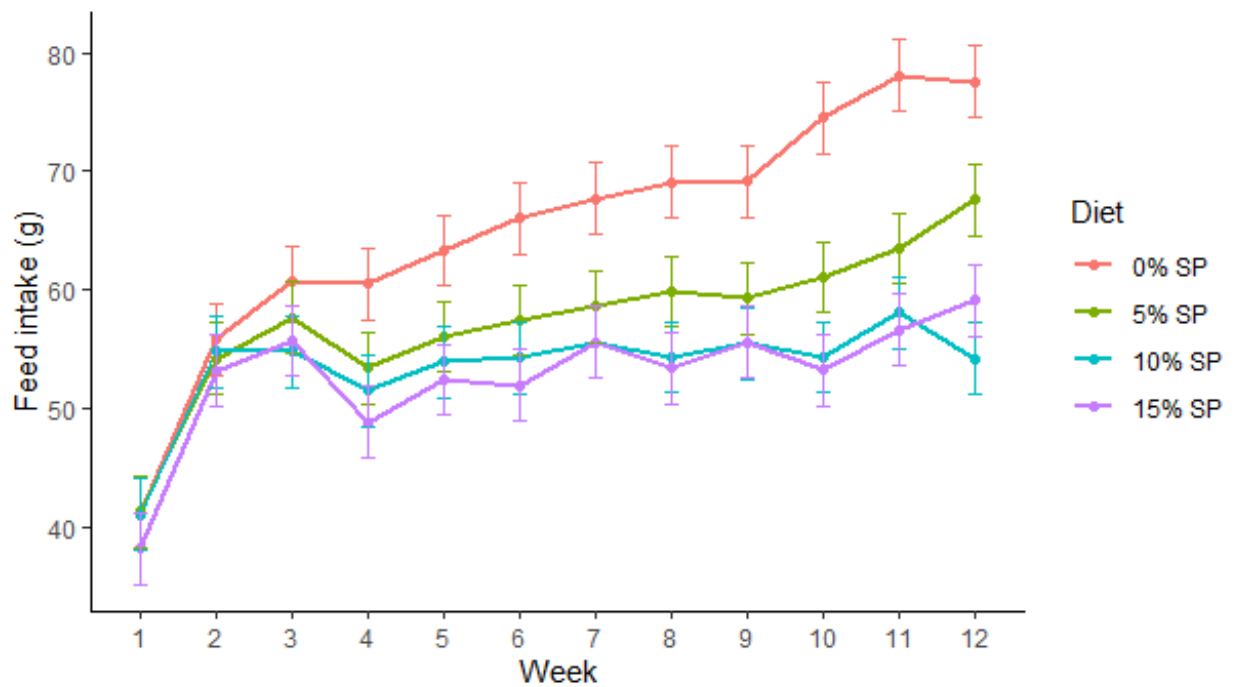
**Table 3.6** Main effects of diet and breed on feed intake parameters of Boschveld and Potchefstroom Koekoek chickens

<b>Diet</b>	<b>FI</b>	<b>PI</b>
0% SP	65.33 <sup>a</sup>	11.79
5% SP	57.56 <sup>b</sup>	11.91
10% SP	53.60 <sup>c</sup>	10.98
15% SP	52.88 <sup>c</sup>	11.43
SEM	2.2530	0.4631
p-value	0.0046	0.4589
<b>Breed</b>		
BV	58.41	11.79
PK	56.27	11.27
SEM	1.5931	0.3275
P-value	0.3563	0.2515

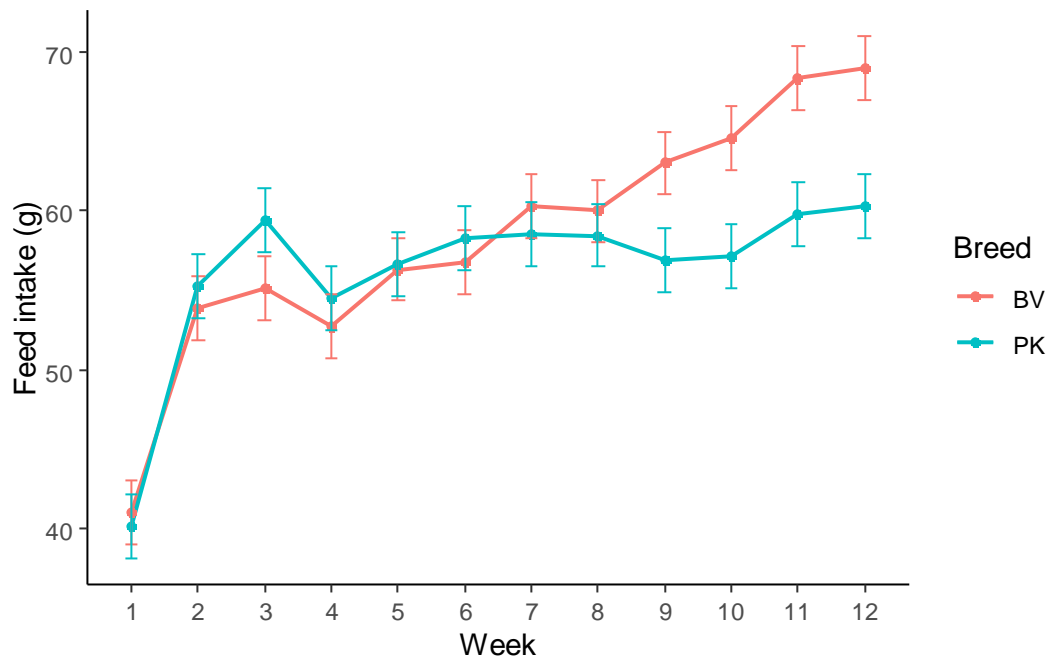
<sup>abc</sup>Means with different superscripts within a column differ ( $P < 0.05$ )

A significant ( $P < 0.05$ ) interaction between dietary treatment and week of measurement on daily feed intake was observed as shown in Figure 3.1. The study results show that all the dietary treatments (0% SP, 5% SP, 10% SP and 15% SP) were consumed in similar quantities ( $P > 0.05$ ) during week 1 to week 3. However, from week 4 up to the end of the study in week 12, 0% SP was consumed in greater quantities ( $P < 0.05$ ) than 5% SP, 10% SP, and 15% SP. The consumption of diets 5% SP, 10% SP and 15% SP did not differ significantly ( $P > 0.05$ ) between week 1 to week 9, but diet 5% SP was consumed in a greater amount ( $P < 0.05$ ) than 10% SP and 15% SP during week 10 and week 12. The diets 5% SP, 10% SP and 15% SP were consumed in similar quantities during week 11. The breed of chickens interacted with weeks of growth to affect ( $P < 0.05$ ) daily feed intake. The feed intakes for both breeds did not ( $P > 0.05$ ) differ significantly at week 1 and week 2 (Figure 3.2). However, the PK breed consumed more

feed ( $P < 0.05$ ) than BV breed at week 3. During the growth weeks 4 to 8, the two breeds had similar quantities ( $P > 0.05$ ) of daily feed intake. The BV breed consumed more feed ( $P < 0.05$ ) than PK from week 9 to week 12 and this contributed to the observed breed x week interaction.



**Figure 3.1** Daily feed intake per week under varying inclusion levels of Spirulina in grower diet.

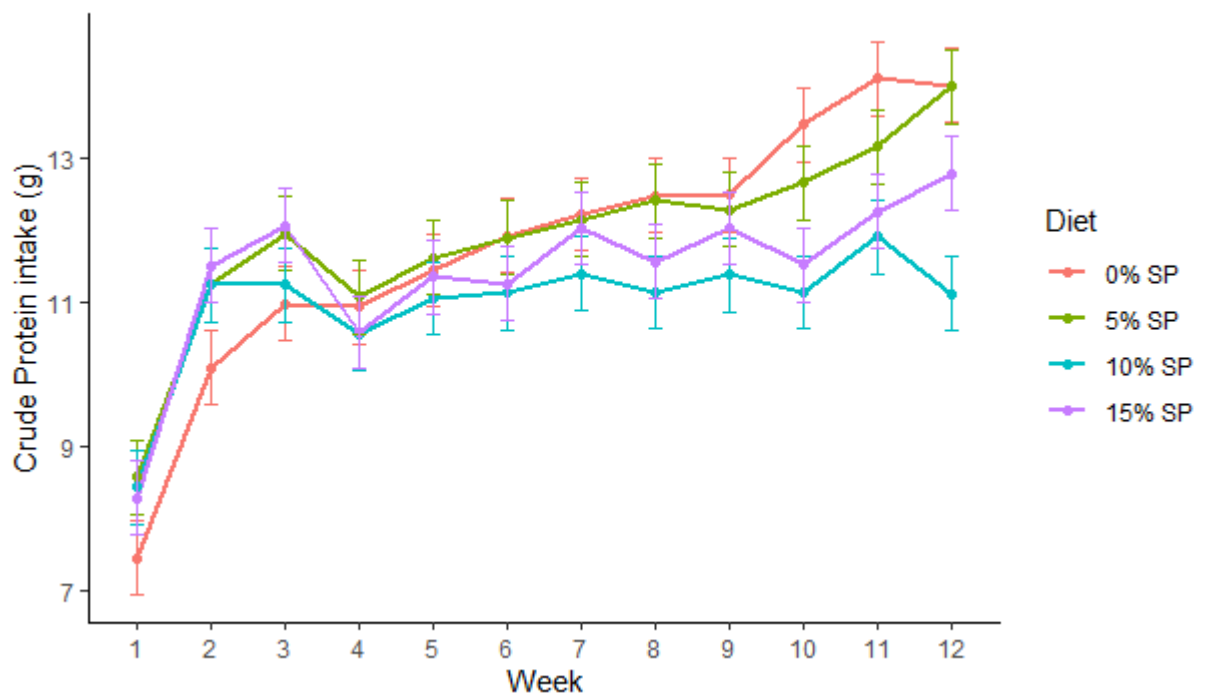


**Figure 3.2** Effects of breed x week interaction on feed intake of Boschveld and Potchefstroom Koekoek chickens fed incremental levels of Spirulina.

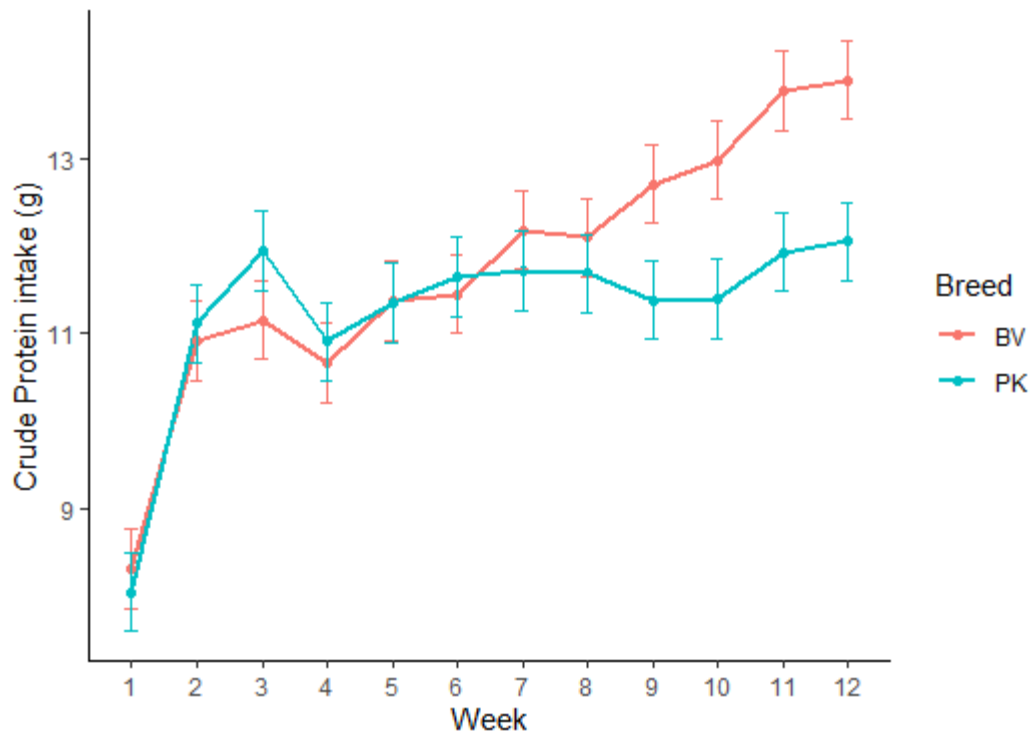
### 3.3.2. Protein intake

The treatment diets interacted with breeds to affect ( $P < 0.05$ ) protein intake. There was no significant difference ( $P > 0.05$ ) between the two chicken breeds in 0% SP and 5% SP. However, 0% SP and 5% SP of both breeds had higher ( $P < 0.05$ ) protein intake than 10% SP and 15% SP (Table 3.5). The BV chickens had a higher protein intake ( $P < 0.05$ ) in diet 15% SP compared to PK chickens. The treatment diets interacted with week to influence ( $P < 0.05$ ) crude protein intake. A similar protein intake ( $P > 0.05$ ) was recorded in all the four diets (0% SP, 5% SP, 10% SP, and 15% SP) at week 1 and from week 3 to week 8. However, chickens fed 10% SP and 15% SP recorded a lower ( $P < 0.05$ ) protein intake during week 10 compared to chickens on diet 0% SP and 5% SP which did not

differ ( $P < 0.05$ ) (Figure 3.3). During week 12, chickens on diet 0% SP and 5%SP recorded higher CP intake ( $P < 0.05$ ) than chickens on diet 10% SP and 15%SP. The CP intake for chickens on diet 15% SP was higher ( $P < 0.05$ ) than that of chickens on diet 10% SP, while chickens on diet 10% SP recorded the lowest CP than chickens on other treatments during week 12. The breed of chickens interacted significantly ( $P < 0.05$ ) with week to influence protein intake. Potchefstroom Koekoek chickens and Boschveld chickens had a similar ( $P > 0.05$ ) protein intake from week 1-8. The protein intake of Boschveld exceeded ( $P < 0.05$ ) that of Potchefstroom Koekoek from week 9 to week 12 as shown in Figure 3.4.



**Figure 3.3** Effects of *Spirulina platensis* inclusion level on weekly protein intake (g/day) in the Boschveld and Potchefstroom Koekoek chickens.



**Figure 3.4** Effects of breed and week interaction on protein intake as influenced by inclusion of Spirulina in the diet.

### 3.3.3 Growth performance

#### 3.3.3.1 Average body weights

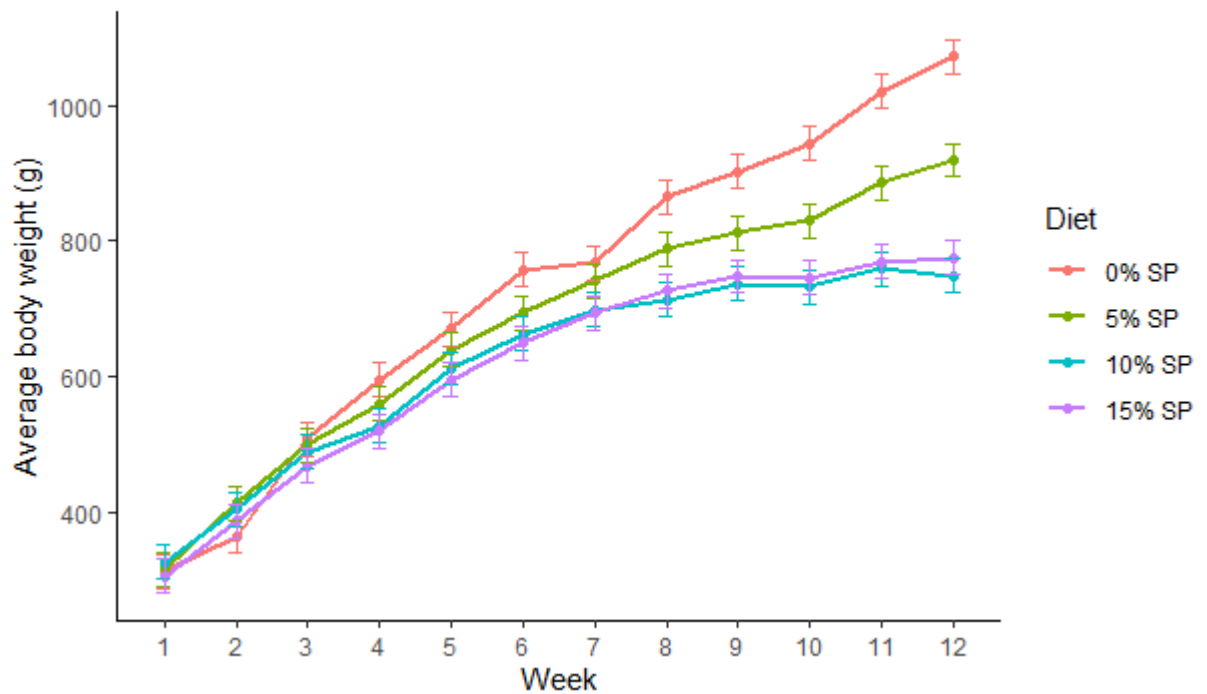
The 3-way interactions diet x breed x week did not affect ( $P>0.05$ ) growth performance parameters (Table 3.7). The average live weight was affected ( $P<0.05$ ) by the interaction of treatment diets with period of growth (Figure 3.5). There was no significant difference ( $P>0.05$ ) in average live weights of the chickens fed the treatment diets

during week 1 to week 5. However, chickens on diet 0% SP had greater ( $P<0.05$ ) live weight than those on 5% SP, 10% SP and 15% SP during week 6 and from week 8 to week 12. Similarly, chickens on diet 5% SP were significantly ( $P<0.05$ ) heavier than chickens on diet 10% SP and 15% SP from week 8 to week 12. However, chickens on diet 10% SP and 15% SP recorded similar ( $P>0.05$ ) live weights throughout the whole experiment.

**Table 3.7** Statistical significance of treatment diets, breed and week, and their interactions on the growth performance as influenced by incremental levels of Spirulina in the diet.

Parameters	Effect of treatment			Interaction			
	Diet	Breed	Week	DxB	DxW	BxW	DxBxW
ABW	***	NS	***	**	***	***	NS
ADG	***	*	NS	NS	NS	NS	NS
FCR	*	NS	***	NS	***	***	NS
PER	***	NS	***	NS	***	*	NS

D= Treatment Diet; B= Chicken breed; W=Week; ABW= Average body weight; ADG= Average daily gain; FCR= Feed conversion ratio; PER= Protein efficiency ratio; \* $P<0.05$ ; \*\* $P<0.001$ ; \*\*\* $P<0.0001$ ; NS= not significant



**Figure 3.5** Effects of dietary treatments and week interaction on average body weights (g) of chickens as influenced by inclusion of Spirulina in the diet.

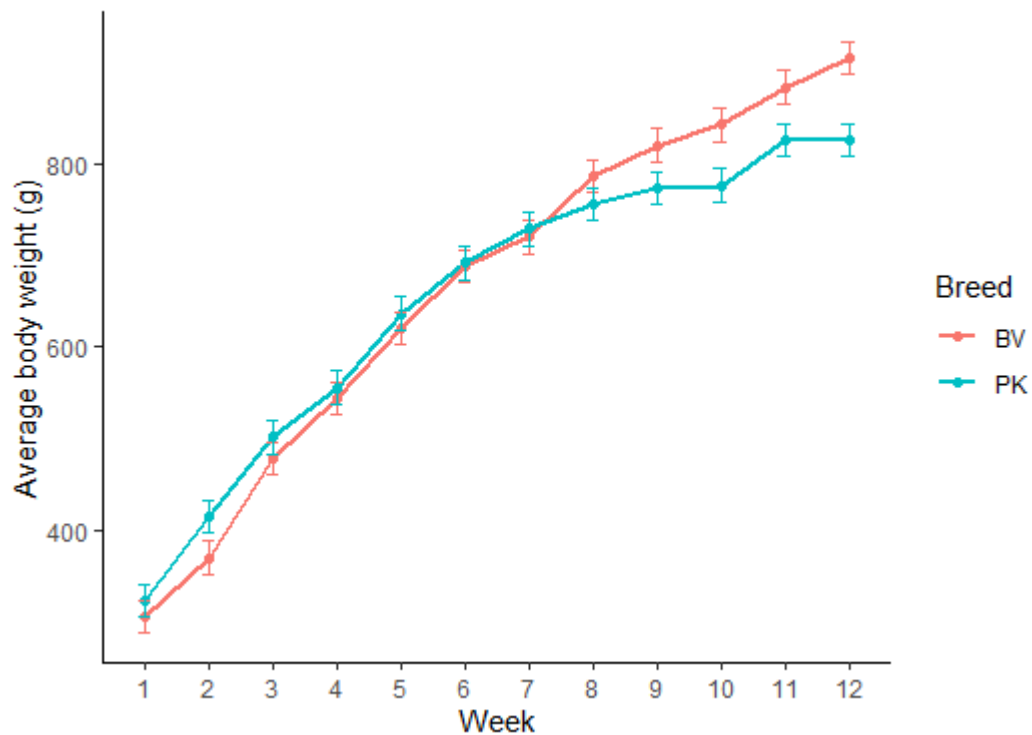
The dietary treatments interacted significantly ( $P < 0.05$ ) with breed to influence live weight as shown in Table 3.8. The PK breed recorded greater live weights ( $P < 0.05$ ) on 0% SP than BV. The average live weights for the BV and PK did not differ significantly ( $P > 0.05$ ) on 5% SP, 10% SP and 15% SP. Thus the differences in live weight between the two breeds in diet 0% SP caused the observed dietary treatment and breed interaction.

**Table 3.8** Effects of treatment diets and breed interactions on growth parameters of Boschveld and Potchefstroom Koekoek chickens.

<b>Breed</b>	<b>Diet</b>	<b>ABW (g)</b>	<b>ADG(g)</b>	<b>FCR</b>	<b>PER</b>
BV	0% SP	671.36 <sup>b</sup>	1.75	0.99	0.95
	5% SP	653.01 <sup>c</sup>	1.68	0.98	0.87
	10% SP	618.69 <sup>c</sup>	1.58	1.02	0.75
	15% SP	585.29 <sup>c</sup>	1.54	1.00	0.62
PK	0% SP	700.43 <sup>a</sup>	1.73	0.97	0.65
	5% SP	637.40 <sup>c</sup>	1.60	1.00	0.56
	10% SP	579.17 <sup>c</sup>	1.40	1.05	0.56
	15% SP	599.56 <sup>c</sup>	1.54	1.01	0.57
SEM		10.4014	0.5632	0.1014	0.0710
P-value		0.0065	0.1209	0.2851	0.4323

<sup>abc</sup>Means with different superscripts within a column differ (P<0.05)

The interaction of chicken breeds and weeks of growth affected ( $P < 0.05$ ) live weights of the birds (Figure 3.6). The Potchefstroom Koekoek chickens had greater live weights ( $P < 0.05$ ) at week 2 than Boschveld chickens. The two breeds did not differ in their live weights at week 1 and from week 3 to week 8 of growth. However, an increase in live weights of Boschveld chickens was observed at week 9 and week 12 where it exceeded that of Potchefstroom Koekoek.



**Figure 3.6** Effects of breed and week interactions on average body weights of chickens fed incremental Spirulina.

**Table 3.9** Main effects of treatment diets and breed on growth parameters of Boschveld and Potchefstroom Koekoek chickens

<b>Diet</b>	<b>ABW</b>	<b>ADG</b>	<b>FCR</b>	<b>PER</b>
0% SP	685.74 <sup>a</sup>	1.74 <sup>a</sup>	0.98 <sup>b</sup>	0.91 <sup>a</sup>
5% SP	645.16 <sup>b</sup>	1.64 <sup>b</sup>	0.99 <sup>b</sup>	0.68 <sup>b</sup>
10% SP	598.60 <sup>c</sup>	1.49 <sup>c</sup>	1.03 <sup>a</sup>	0.61 <sup>c</sup>
15% SP	592.35 <sup>c</sup>	1.54 <sup>c</sup>	1.01 <sup>a</sup>	0.57 <sup>c</sup>
<b>SEM</b>	7.3549	0.3984	0.0717	0.052
<b>p-value</b>	0.0001	0.0001	0.0142	0.0002
<b>Breed</b>				
BV	631.25	1.64 <sup>a</sup>	1.00	0.67
PK	627.47	1.57 <sup>b</sup>	1.01	0.57
<b>SEM</b>	5.2007	0.2817	0.0508	0.037
<b>P-value</b>	0.6192	0.0119	0.2771	0.1179

<sup>abc</sup>In a column, means with different superscripts differ (P<0.05)

### 3.3.3.2. Average daily gain (ADG)

The average daily gain of the chickens was influenced by diet and breed. The Boschveld chickens had higher (P<0.05) ADG than Potchefstroom Koekoek chickens (1.64g vs 1.57g). Within the diets, chickens on diet 0% SP recorded higher (P<0.05) ADG (1.74g) compared to chickens on diet 5% SP (1.64g). However, chickens on diet 10% SP and 15% SP had similar (P>0.05) ADG of 1.49g and 1.54 g, respectively as shown in Table 3.9 above.

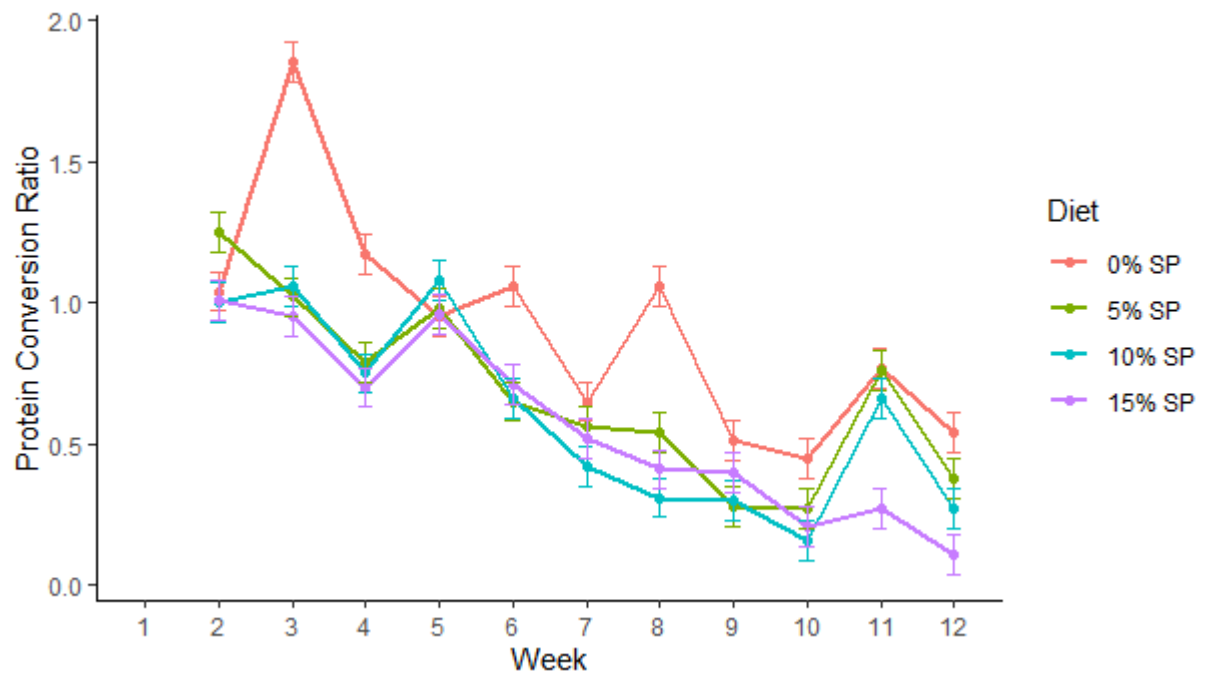
### 3.3.3.3. Feed conversion ratio (FCR)

The 3-way interactions of breed x diet x week and the 2-way interactions; breed x diet, breed x week and diet x week did not (P>0.05) influence the FCR of the chickens (Table

3.7). However, diet significantly ( $P<0.05$ ) influenced the FCR of the chickens. A high FCR ( $P<0.05$ ) was observed in chickens offered diet 10% SP and 15% SP. Chickens on diet 5% SP and 0% SP had similar ( $P>0.05$ ) FCR, which was lower ( $P<0.05$ ) than that of 10% SP and 15% SP as given in Table 3.9. The period of growth also influenced ( $P<0.05$ ) the FCR. Except for week 2, a low FCR was observed between week 3 and week 8, while an increase in FCR was recorded from week 9 to week 12.

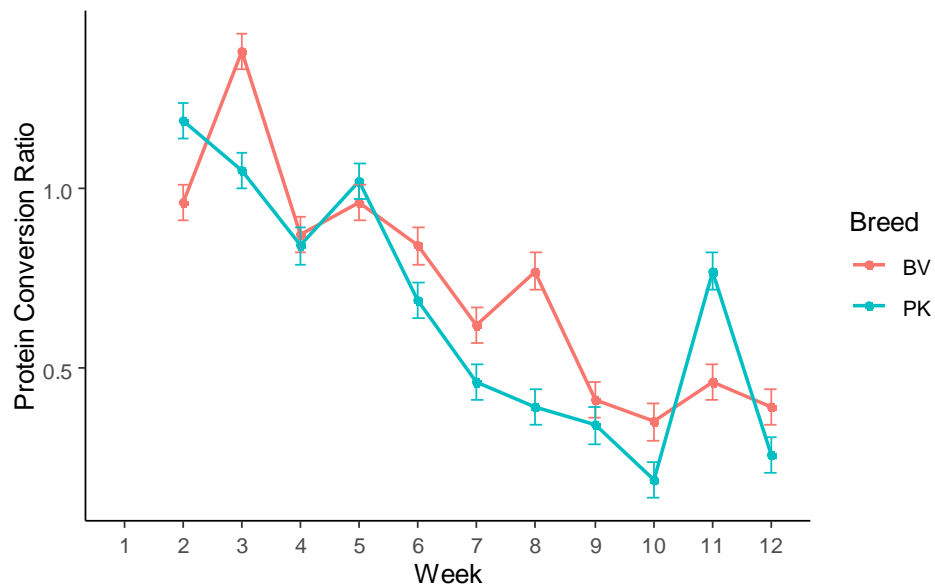
#### **3.3.3.4. Protein efficiency ratio (PER)**

The 3-way interactions of breed x diet x week and two-way interaction of dietary treatment by breed did not ( $P>0.05$ ) influence the PER (Table 3.7). Treatment diet and week interacted significantly ( $P<0.05$ ) to influence PER. Except for chickens on diet 5% SP, the chickens on 0% SP, 10% SP and 15% SP had similar ( $P>0.05$ ) PER at week 2. Chickens on 5% SP recorded high ( $P<0.05$ ) PER at week 2. The chickens on 0% SP had high PER ( $P<0.05$ ) at week 3, week 6, week 8 and week 10 than that of chickens on other treatment diets. Chickens fed diet 15% SP recorded low PER ( $P<0.05$ ) from week 11 to week 12. Birds on 5% SP and 10% SP had similar ( $P>0.05$ ) PER from week 3 to week 12 (Figure 3.7).



**Figure 3.7** Effects of *Spirulina platensis* inclusion levels on weekly protein efficiency ratio of Boschveld and Potchefstroom Koekoek chickens.

The chicken breed interacted with period of growth (week) to influence ( $P < 0.05$ ) protein efficiency ratio (Figure 3.8). The PK breed only recorded high ( $P < 0.05$ ) PER at week 2 and week 11. The BV breed recorded high ( $P < 0.05$ ) PER at week 3, week 6, week 7, week 8, week 10 and week 12. Both breed BV and PK recorded similar ( $> 0.05$ ) PER during week 4, week 5 and week 9.



**Figure 3.8** Effects of chicken breed and period of growth (week) on protein efficiency ratio.

### **3.4. DISCUSSION**

#### **3.4.1. Feed intake**

The inclusion of Spirulina in the current study resulted in reduced feed intake of the chickens offered Spirulina diets than those on control diet and this varied with level of inclusion and week of measurement. The low feed intake in chickens fed Spirulina based diet may be attributed to the high amount of phenolic compounds found in Spirulina diets than in control. High concentration of tannins have been shown to have anti-nutritional effects on monogastric animals, with poultry reported to be more sensitive (Suleyman, 2017) and tannins causes an astringent taste of the feed which lead to low palatability and decreases feed intake (Mahfuz *et al.*, 2021). Spirulina is also known to have weak organoleptic properties causing undesirable flavor which affects palatability and may discourage feed consumption (Rajmohan, 2017 & Hamdan *et al.*, 2020). The flavor of a feed affects feed intake as birds are more responsive to weakly flavored feed than strongly flavored feed as the bird's sense of taste can be overwhelmed (Gentle, 1985) which may explain the reduced feed intake in this study. These results are in agreement with those of Gongnet *et al.* (2001) & Zahir *et al.* (2019) who reported reduced feed intake with increasing Spirulina concentration in diets of broilers. The current study results contradict those of Mirzaie *et al.* (2018), Pestana *et al.* (2020) & Mullenix *et al.* (2022) who supplemented broilers with Spirulina at the inclusion levels similar to that of this study (10% and 15% Spirulina) and reported no significant difference in the feed intake of broiler chickens fed control diet and those fed Spirulina diets. This study results also contradict that of Ansari *et al.* (2018) & Khan *et al.* (2020) who reported high feed intake in broiler chickens supplemented with

Spirulina at 2g/kg than the control group, they associated the recorded high in feed intake in Spirulina group to its good taste, odor, and pH, and its high digestibility which reduces acidity and constipation. Different results could be attributed to factors such; the processing method of the Spirulina, the use of different chicken breeds in the studies, as the strain of a chicken affects its performance including feed intake (Ikusika *et al.*, 2020 & Gafar *et al.*, 2022) and different inclusion levels of Spirulina used and the production systems.

Feed intake significantly changed with time (weeks) as the birds got used to the experimental diets. An increase in weekly feed intake within the dietary treatment was observed as expected with increasing age of birds, because as chickens increase in size, their nutritional requirements also increase and they eat to satisfy their needs (Sebola, 2015). The variation in feed intake across the weeks in the dietary treatments could be attributed to the concentration of the Spirulina in the diets. The chickens happened to have consumed more of control diet 0% SP and 5% SP over the weeks than 10% SP and 15% SP which was consumed less but in similar quantities from week 3 to week 11. Body size may also explain the differences in feed intake across the weeks in the two chicken breeds, in which PK which is generally heavier than BV is observed to have consumed more feed in the early weeks than the BV breed. BV chickens however consumed more feed in the last half of the experimental period which may indicate better adaptation of the Spirulina diet by the BV at an older age than the PK. This study results agrees with the view that the genetics of a chicken influences its feed consumption at different stages (Rondelli *et al.*, 2003).

### **3.4.2. Protein intake**

The higher CP concentration in Spirulina affects the CP intake of the birds. The *Spirulina platensis* was high in CP (71.2%); the CP was higher than the values reported by other authors (Sarker *et al.*, 2016, Khalila *et al.*, 2019 & Zahir *et al.*, 2019). The differences could be attributed to the quality of Spirulina used. In the present study, it was observed that increasing the level of Spirulina in the diet appears to have increased the crude protein concentration of the diet, which resulted in a corresponding decrease in the age at which peak CP intake, was reached. Birds offered diets containing Spirulina (5% SP, 10% SP & 15% SP) tend to have reached their peak protein intake at an early age than those on control diet which can be associated with observed low protein content in the control diet than in the Spirulina diet. The differences in protein intake among the breeds and period of growth when fed in this study can be attributed to genetic variations in terms of nutrients requirements and utilization of the two breeds with age (Sebola, 2015). As far as the cost of Spirulina is concerned, it is evidence that inclusion of Spirulina in grower diets at high percentage can only be beneficial up to a certain age because birds fed high level of Spirulina will reach peak intake at an early stage and thereafter the protein intake will decrease which may suggest that Spirulina may be better off as a supplement.

### **3.4.3. Average body weights and average daily gain**

Chickens fed control diet (0% SP) recorded heavy body weights per week compared to chickens fed Spirulina diets. This may be attributed to high feed intake per week in birds on diet 0% SP than those on diets containing Spirulina. The results from this study

agrees with those of Gongnet *et al.* (2001), Ross (1990) and Pestana *et al.* (2020) as they reported lower body weight in birds fed Spirulina diets in comparison to birds fed control diet. In contrast, the results disagree with those of Bonos *et al.* (2015), Mariey *et al.* (2012) & Sugiharto *et al.* (2022) that reported a non-significant difference in the body weight between birds supplemented Spirulina diets and control group. Feed intake is positively correlate to growth performance of meat-type poultry because it is the main factor that influences body weight gain (Ferket & Gernat, 2006). Overall low body weights from this study might be linked to lower feed intake in chickens fed Spirulina diets and poor feed conversion ratio in birds fed Spirulina diet than control.

BV chickens however consumed more feed and had more protein intake in the last half of the experimental period hence more energy and nutrients were available for growth in the BV during that time, which may have contributed to the higher ADG in the BV compared to the PK.

#### **3.4.4. Feed conversion ratio**

The FCR from this study indicates that the ability to utilize the Spirulina containing diet varied among the dietary treatments and period of growth (weeks). High FCR was recorded in chickens fed diet 10% SP and 15% SP than in birds fed 5% SP and 0% SP diet which mean the increasing level of Spirulina in the diet resulted in more feed needed to be consumed for the chickens to convert the feed into body weight. The difference in FCR of chickens among the diets is attributed to the observed difference in weights of the chickens on these diets as. Chickens on diet 0%SP and 5% SP had more weight compared to those on diet 10% SP and 15% SP which proves that FCR is

negatively correlated to body weight gain (Chen *et al.*, 2021). These results are in agreement with those of Gongnet *et al.* (2001), Mullenix *et al.* (2022), Pestana *et al.* (2020) & Zahir *et al.* (2019), who reported high feed conversion ratio in chickens fed Spirulina than control group. However, these contradict those of Sugiharto *et al.* (2022) and Mirzaie *et al.* (2018) who used Spirulina in their feeding trials with chickens and reported no significant differences in FCR of all treatments. Contradictory results are possible due to different inclusion levels of Spirulina, the quality and different chicken breeds used in the present study compared to the other studies. It may also be attributed to secondary parameters such as feed composition, environmental conditions such as temperature in which the studies were conducted.

#### **3.4.5. Protein efficiency ratio**

The protein efficiency ratio (PER) declined with age of the birds in both breeds. The decline in PER with age is expected due to faster growth in young animals up the inflection point on the growth curve when they attain their maximum growth rate (Hosseini-Zadeh, 2015). The chickens on Spirulina diet had low PER compared to control group, which could be attributed to the high amount of tannins in Spirulina diet which affected the digestibility of protein and resulted in higher energy expenditure to excrete the products of protein metabolism in the Spirulina diets which had higher crude protein concentration (Gous *et al.*, 2018).

Overall, *Spirulina platensis* is known to have poor organoleptic properties (Rajmohan, 2017; Parwani & Singh, 2018) that limit its acceptance and restrict its use in animal feeds. Additionally, Spirulina is noted to have a bitter taste (Rajmohan, 2017) and a

fishy or oceanic scent (Hadman *et al.*, 2020) that gives off-flavors which negatively affects its palatability and consequently feed intake and growth performance of animals when incorporated into the feeds. All these properties may explain the low feed intake and poor weight gains recorded in the current study.

### **3.5. Conclusion**

Replacing soybean with Spirulina at 10% - 15% in the chicken diets negatively affected feed intake, average body weights, average daily gain, feed conversion ratio and protein efficiency ratio. From this study, it is evidence that it is only safe to replace soybean with Spirulina as a protein source at about 5% per 100kg of feeds, beyond that it has detrimental effect on growth performances of chickens. However, when chickens consume diets containing Spirulina, they reach their peak protein intake much earlier which indicates that Spirulina has high amount of protein. Additionally, high levels (10-15%) of Spirulina in the diet may induce unfavorable FCR which means more feed will be required per unit body weight gain in chickens which will increase the cost of feeding chickens.

### **3.6. Recommendations**

This study recommends that, for further studies on the growth performance of chickens the sex of the birds must be considered as it might have an effect on the growth performance. The study further recommends analyses of anti-nutritional factors of *Spirulina platensis* that might be capable of affecting feed intake and growth performance parameters of chickens and other animals when Spirulina is to be used as animal feed. The study also recommends that, although Spirulina contains high protein

content, it must only be used in a small amount in the diet to alleviate most of the production problems such as, low feed intake and poor growth performance.

### 3.7. References

- Akinola, L. A. F., & Essien, A. (2011). Relevance of rural poultry production in developing countries with special reference to Africa. *World's Poultry Science Journal*, 67: 697-705.
- Ansari, M. S, Hajati, H., Gholizadeh, F., Soltani, N.,& Alavi, M. (2018). Effect of different levels of spirulina platensis on growth performance, intestinal morphology, gut microflora, carcass characteristics and some blood parameters in broiler chickens. *Journal of Phycological Research*, 2(2): 187-197.
- Association of the Official Analytical Chemists (AOAC). (2016). Official Methods of Analysis of AOAC international. 20<sup>th</sup> Edition.
- Bagopi, E., Chokwe, E., Halse, P., Hausiku, J., Humavindu, M., Kalapula, W., & Roberts, S. (2016). Competition, agro-processing and regional development: The case of the poultry section in South Africa, Botswana, Namibia and Zambia. *Competition in Africa: Insights from Key Industries*, 66-101.
- Bonos, E., Kasapidou, E., Kargopoulos, A., Karampampas, A., & Christaki, E. (2016). *Spirulina* as a functional ingredient in broiler chicken diets. *South African Journal of Animal Science*, 46(1):95-102.
- Boling-Frankenbach, S.D., Peter, C. M., Douglas, M. W, Snow, J. L., Parsons, C. M, & Baker, D. H. (2001). Efficacy of phytase for increasing protein efficiency ratio (PER) values of feed ingredients. *Poultry Science*, 80:1578-1584.
- Chen, C., Su, Z., Li, Y., Luan, P., Wang, S., Zhang, H., ... & Leng, L. (2021).

- Estimation of the genetic parameters of traits relevant to feed efficiency: result from broiler lines divergent for high or low abdominal fat content. *Poultry Science*, 100(2): 461-466.
- Despins, J. C and Axtell, R.C. (1995). Feeding behavior and growth of broiler chicks fed larvae of the darkling beetle, *Aphitobius diaperinus*. *Poultry Science*, 74: 331-336.
- Draaisma R., Wijffels R., Slegers P. & Brentner L. (2013). Food commodities from microalgae. *Current Option in Biotechnology*, 24: 169-177.
- Gafar, E. G., Abdo, S. G., Mahrous, M. Y., & Kamal, M. A. (2022). Effect of strain and sex on productive performance and carcass traits in some broiler chickens. *Archives of Agriculture Sciences Journal*, 5(1): 77-78.
- Gongnet, G. P., Niess, E., Rodehutschord, M., & Pfeffer, E. (2001). Algae-meal (*Spirulina platensis*) from lake Chad replacing soybean-meal in broiler diets. *Archiv Fur Geflugelkunde*, 65 (6): 265-268.
- Gous, R. M., Faulkner, A. S., & Swatson, H. K. (2018). The effect of dietary energy: protein ratio, protein quality and food allocation on the efficiency of utilisation of protein by broiler chickens. *British poultry science*, 59(1), 100-109.
- Gentle, M.J. (1985). Sensory involvement in the control of food intake in poultry. *Proceedings of the Nutrition Society*, 44: 313-221.
- Ferret, P.R., and Gernat, A.G. (2006). Factors that affect feed intake of meat birds: A review. *International Journal of Poultry Science*, 5(10) :905-911.
- Hamdan, A. B., Riady, C., Fitriya, W., & Ekantari, N. (2020). Effects of

nanoencapsulated carotenoid of *Spirulina platensis* on the sensory profiles of dark and milk chocolate. In *E3S Web of Conferences* (Vol. 147, p. 03022). EDP Sciences.

Hosseini-Zadeh, N. G. (2015). Modeling the growth curve of Iranian Shall sheep using non-linear growth models. *Small Ruminant Research*, 130: 60-66.

Ikusika, O. O., Falowo, A. B., Zindove, T. J., & Okoh, A. I. (2020). Effect of strain, sex and slaughter weight on growth performance, carcass yield and quality of broiler meat. *Open Agriculture*, 5(1): 607-616.

Khalila, H. S., Fayed, W. M., Mansour, A. T., Srour, T. M., Omar, E. A., Darwish, S. I., & Moussa Nour, A. A. (2019). Dietary Supplementation of *Spirulina* (*Arthrospira platensis*), With Plant Protein Sources and their Effects on Growth, Feed Utilization and Histological Changes in Nile Tilapia, *Oreochromis niloticus*. *Journal of Aquaculture Research & Development*, 9: 549.

Khan, S., Mobashar, M., Mahsood, F. K., Javaid, S., Abdel-Wareth, A. A., Ammanullah, H., & Mahmood, A. (2020). Spirulina inclusion levels in a broiler ration: evaluation of growth performance, gut integrity, and immunity. *Tropical Animal Health and Production*, 52: 3233-3240.

Littell, R. C., Henry, P. R., & Ammerman, C. B. (1998). Statistical analysis of repeated measures data using SAS procedures. *Journal of animal science*, 76(4), 1216-1231.

Lordan, S., Ross, R. P., & Stanton, C. (2011). Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Marine*

*drugs*, 9(6): 1056-1100.

- Magothe, T. M., Okeno, T. O., Muhuyi, W. B., & Kahi, A. K. (2012). Indigenous chicken production in Kenya : II . *Prospects for Research and Development*, 68: 133–145.
- Mahfuz, S., Shang, Q., & Piao, X. (2021). Phenolic compounds as natural feed additives in poultry and swine diets: A review. *Journal of Animal Science and Biotechnology*, 12(1): 1-18.
- Masaire, E., Madzingira, O., Samkange, A., Kandiwa, E., Mushonga, B., & As, B. (2018). Characterization of poultry production and management systems in the communal areas of Namibia. *African Journal of Poultry Farming*, 6(3): 265–276.
- Mariey, Y. A., Samak, H. R., & Ibrahim, M. A. (2012). Effect of using *Spirulina platensis* algae as a feed additive for poultry diets: 1-productive and reproductive performances of local laying hens. *Egyptian Poultry Science Journal*, 32(1): 201-215.
- Makkar, H. P. (2003). Measurement of total phenolics and tannins using Folin-Ciocalteu method. *Quantification of tannins in tree and shrub foliage: A Laboratory Manual*, 49-51.
- Mirzaie, S., Zirak-khattab, F., Hosseini, S. A., & Donyaei-darian, H. (2018). Effects of dietary *Spirulina* on antioxidant status , lipid profile , immune response and performance characteristics of broiler chickens reared under high ambient temperature. *Asian-Australasian Journal of Animal Sciences*, 31(4): 556–563.

- Mullenix, G. T., Maynard, C. J., Owens, C. M., Rochell, S. J., Bottje, W. G., Brister, R. D., & Kidd, M. T. (2022). Spirulina platensis meal inclusion effects on broilers fed a reduced protein diet. *Journal of Applied Poultry Research*, 31: 100199.
- Packard, R. (2014). *Comparison of production parameters and meat quality characteristics of South African indigenous chickens* (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Park, J. H., Lee, S. I., & Kim, I. H. (2018). Effect of dietary Spirulina (*Arthrospira*) platensis on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission and breast meat quality of broiler chickens. *Journal of Poultry Science*, 97:2451-2459.
- Parwani, L., & Singh, J. (2018). Effect of temperature on nutritional values of Spirulina: Useful for nutrient sustainable food preparations to combact malnutrition. *Biological Science*, 89(4): 1259-1265.
- Petrus, N. P. (2011). *Characterisation and production performance of indigenous chickens in northern Namibia regions*. PhD dissertation. University of Namibia, Namibia.
- Pestana, J. M., Puerta, B., Santos, H., Madeira, M. S., Alfaia, C. M., Lopes, P. A., ... & Prates, J. A. M. (2020). Impact of dietary incorporation of Spirulina (*Arthrospira* platensis) and exogenous enzymes on broiler performance, carcass traits, and meat quality. *Poultry science*, 99(5): 2519-2532.
- Poppi, D. P., & McLennan, S. R. (2010). Nutritional research to meet future challenges.

*Animal Production Science*, 50: 329-338.

Rajmohan, D. K. D. (2017). *Use of Ionic Gelation to Reduce Perceived Bitterness of Spirulina Protein* (Doctoral dissertation, Oklahoma State University).

Rondelli, S., Martinez, O., Garcia, P.T. (2003). Sex effect on productive parameters, carcass and body fat composition of two commercial broiler lines. *Brazil Journal of Poultry Science*, 5: 169-173.

Ross, E., & Dominy, W. (1990). The nutritional value of dehydrated, blue-green algae (*Spirulina plantensis*) for poultry. *Poultry science*, 69(5): 794-800.

Sarker, P. K., Gamble, M. M., Kelson, S., & Kapuscinski, A. R. (2016). Nile tilapia (*Oreochromis niloticus*) show high digestibility of lipid and fatty acids from marine *Schizochytrium* sp. and of protein and essential amino acids from freshwater *Spirulina* sp. feed ingredients. *Aquaculture Nutrition*, 22(1): 109–119.

Sebola, N. A. (2015). A nutritional assessment of *Moringa oleifera* leaf meal for chickens commonly reared under extensive production systems: Effect on growth performance, serum biochemistry, and meat quality. Doctoral dissertation, North-West University (South Africa).

Sugiharto S, Atmaja BM, Widiastuti E, Hadiyanto H. (2022). Combined use of *Spirulina platensis* and *Saccharomyces cerevisiae*: Implication on growth, blood profile and intestinal morphology and bacteria of the Indonesian crossbred chickens. *Biodiversitas*, 23: 160-165.

Suleyman, C. (2017, May). Tannins and their effects on poultry nutrition.

In *International Conference Agriculture, forestry, food Science and Technology. Cappadocia, Turkey* (pp. 15-17).

Van Marle-Koster, E., & Webb, E. C. (2000). Carcass characteristics of South African native chicken lines. *South African Journal of Animal Science*, 30(1): 53-56.

Van Soest, P. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of dairy science*, 74(10): 3583-3597.

Zahir, U. R., Md. Anwarul, H. B., Maksuda, B., & Noushin, A. (2019). Effects of dietary supplement of dried Chlorella powder as an alternative to antibiotic on growth performance and health status of broiler chicken. *International Journal of Biosciences*, 14 (4): 255-263.

## CHAPTER 4

### **EFFECTS OF FEEDING INCREMENTAL LEVELS OF *SPIRULINA PLATENSIS* ON CARCASS YIELD AND INTERNAL ORGAN SIZES OF BOSCHVELD AND POTCHEFSTROOM KOEKOEK CHICKEN BREEDS**

#### **Abstract**

The study investigated the effect of feeding graded levels of *Spirulina platensis* in grower diets on the carcass yield, carcass components and internal organ sizes of Boschveld and Potchefstroom Koekoek chickens. A total of 72 one-day old unsexed indigenous chicks consisting of 36 Boschveld and 36 Potchefstroom Koekoek were used in the experiment. For the first 4 weeks, the chicks were fed with a commercial broiler starter diet alone. At week five, the chickens were randomly allocated to the four treatment diets (0% SP control; 5% SP; 10% SP and 15% SP Spirulina inclusion level) and were offered the experimental diets for twelve weeks. Each treatment consisted of nine chickens per breed with three replicates of three chickens per cage. At the end of the feeding period a total of 24 chickens, six per treatment, were slaughtered. The thigh, breast, drumstick, wing, gizzard, intestine, liver, heart and spleen were weighed, recorded among the dietary treatments and breeds. The effect of dietary Spirulina on carcass cuts yield and internal organ sizes of the two chicken breeds (Potchefstroom Koekoek and Boschveld) were measured and the data was analyzed using the general linear model (GLM) procedure in SPSS for a two-way analysis of variance (ANOVA) for a 4 (diets) x 2 (chicken strains) factorial treatment arrangement. The results showed

that dietary treatment x breed interactions did not ( $P>0.05$ ) affect the gizzard, spleen, heart, liver and intestines weights and carcass cuts yields. Dietary treatment had a significant ( $P<0.05$ ) effect on the thigh and breast weights of the breeds but did not ( $P>0.05$ ) affect slaughter weight, dressing weight, dressing %, drumstick and wing weights. Breast muscles and thigh weight decreased with the increase in Spirulina inclusion level in the grower diet. The inclusion of Spirulina as a replacement of soybean at different inclusion levels did not ( $P>0.05$ ) affect gizzard, spleen, heart, liver and intestine weights of the chickens. In addition, the breed did not ( $P>0.05$ ) influence any of the carcass cuts yield, gizzard, heart, liver and intestine weights but significantly ( $P<0.05$ ) affected the spleen weight. In conclusion, from the results of this study Spirulina can replace soybean meal at 5% level of inclusion without negatively affecting the carcass yield, carcass components and internal organs of the BV and PK chickens.

**Key words:** diet, breed, carcass, organs, weights

#### **4.1. Introduction**

Carcass attributes are essential in identifying portion yields for commercial purpose, this includes commercial cuts such as thigh, wing, drumstick, breast, neck and feet/shank. However, carcass characteristics parameters include parameters such as live slaughter weight, hot carcass weight, cold carcass weight and dressing percentage (Omojola *et al.*, 2004). Cold carcass weight is the heaviness of the carcass after 24 hours of cooling, while hot carcass weight is the carcass heaviness after slaughter without the head, internal organs and intestines. Carcass characteristics are affected by many factors including amino acid content of the diet which support normal development in broiler chickens (Uushona, 2015). Sebola (2015) reported that the dressing weight, carcass weight, drumstick, thigh and wing weights are influenced by the gender of the chicken. Furthermore, Singh & Pathak (2016) reported that hot carcass weight is affected by age and type of breed while cold carcass weight is affected by the stability of refrigeration storage which might be due to higher muscle activities in the chicken breed used.

Cabrol *et al.* (2021) stated that, the slaughter weight, dressing weight, cuts and percent yield of breast, thigh and drumstick tissues are dependent on rearing system than on broiler sex. Meat from indigenous chickens that are raised in extensive system is known to be tough, lean and flavorful (Packard, 2014). Slower growing chickens have an advantage of producing meat with less fat, a more desirable meat composition as well as a good proportion of retail cuts (Packard, 2014). However, the performance of these birds is low due to inadequate nutrition and vulnerability to diseases among many other factors (Masaire *et al.*, 2018). However, strategies to improve performance of these

breeds needs to be explored (Pestana *et al.*, 2020). High quality diet can be supplied to increase the growth performance. Soybean meal is the major protein source of poultry feed which is scarce in (Hafeni *et al.*, 2013) and this challenges poultry production. It is therefore necessary to find suitable alternative sources of protein such as microalgae (i.e. *Spirulina platensis*) Kovač *et al.* (2013). *Spirulina* is rich in proteins for animals and humans (Mirzaire *et al.*, 2018). In poultry, it improves growth performance as well as carcass quality (Park *et al.*, 2018).

The utilization of microalgae as feed ingredient can contribute to the safety of the environment and natural resources, specifically land degradation and water deprivation (Pestana *et al.*, 2020). *Spirulina platensis* is blue-green algae, edible, high in protein and able to survive in harsh environment and has gained popularity in exploration as food and feed. Dietary *Spirulina* has been linked with increased growth performance of chickens (Park *et al.*, 2018) and enhanced carcass value (Venkataraman *et al.*, 1994). Studies on the effect of dietary *Spirulina* on internal organ sizes of broiler chickens has shown no significant differences in relative weight of liver (Zahir *et al.*, 2019), gizzard, liver and intestines (Kaoud, 2012). Numerous studies have been conducted using *Spirulina* as functional ingredient in broiler chickens (Ansari *et al.*, 2018). Limited researches were done on indigenous and dual purpose chickens. Therefore, the objective of this study was to determine the effect of incremental levels of *Spirulina platensis* in grower diets on the carcass yield and internal organ sizes of the Boschveld and Potchefstroom Koekoek chickens.

## **4.2. Materials and methods**

### **4.2.1 Experimental design, bird management and diets**

The materials and methods on the bird management and care, experimental design, treatment diet formulation and dietary treatment composition are outlined under Chapter 3 under section 3.2.1, 3.2.2, 3.2.3 and 3.2.4, respectively.

### **4.2.2 Slaughtering process**

At the end of week 12, 24 chickens were selected which included six chickens per treatment. The chickens were handled properly without hurting them. The chickens were taken out of the cages by holding the thighs and wings. The chickens were weighed before slaughter to obtain slaughter weight. The slaughter weight was taken using a DS-673 digital weighing scale (Teracka Seiko CO, LTD, Japan). The chickens were slaughtered according to the procedures highlighted in the guidelines on chicken slaughtering and chicken meat handling (Department of Agriculture, Forestry & Fisheries (DAFF), 2006). The chickens were placed into a cone facing head down, the chicken's head was brought down and was exsanguinated by a unilateral neck cut with a sharp knife that severe the right carotid artery and jugular vein. After bleeding for 3-4 minutes, the chickens were put in water (60°C) for 5 min, this allowed some of the fat to melt around the pin feathers, making it easier to de-feather. After removal of the feathers, the chickens were eviscerated.

#### **4.2.3. Carcass yield and internal organ size measurements**

The carcasses were eviscerated manually and subsequently; the internal organs were removed from the carcass. The dressing weight was measured on the digital weighing scale. After 15 min, the carcass initial pH was taken from the right breast muscle using a Testo 205 pH meter (Testo inc., 40 White Lake Rd, Sparta, USA) and thereafter the carcasses were immediately chilled at 4 °C for 24 h. The internal organs excised were the heart, liver, spleen, intestines and the gizzard. The gizzard was cut open, cleaned and rinsed with clean water. The heart, spleen, liver, gizzards and intestines were weighed individually using a digital weighing scale. The ultimate pH of the right breast muscle was taken 24h after chilling. Afterward, the carcasses were taken to the laboratory for analyses.

The carcasses were divided into retail cuts namely wing, breast, drumstick and thigh, using a sharp deboning knife following a procedure by Mast *et al.* (1982). The carcasses and internal organs were then weighed individually using a DS-673 digital weighing scale (Teracka Seiko CO, LTD, Japan). The breast muscle samples were vacuum-packed and stored in a freezer at -18 °C pending further analyses.

#### **4.2.4. Statistical analyses**

The carcass yield and internal organs data were analyzed using the general linear models (GLM) procedure in SPSS v27 (2020) for two-way analysis of variance (ANOVA) for a 4 (diets) x 2 (chicken strains) factorial treatment arrangement. The linear model employed was as follows:

$$Y_{ij} = \mu + D_i + B_j + (D \times B)_{ij} + E_{ij}$$

Where  $Y_{ij}$  is the observation of the treatment of the dependent variable  $ij$ ,  $\mu$  the fixed effect of population mean variable,  $D_i$  = the effect of  $i^{\text{th}}$  treatment diet (0% SP, 5% SP, 10% SP, 15% SP);  $B_j$  = the effect of  $j^{\text{th}}$  chicken breed (BV, PK);  $(D \times B)_{ij}$  = the interaction effect of the  $i^{\text{th}}$  diet and  $j^{\text{th}}$  chicken breed;  $E_{ij}$  = the random error. Significance level was set at  $P < 0.05$ . Means indicating significant differences were tested using Duncan`s multiple range test.

## 4.4. Results

### 4.4.1. Carcass yield

The results of carcass yield of the two chicken breeds (Boschveld and Potchefstroom Koekoek) fed grower diet with four different inclusion levels of Spirulina are presented in Table 4.1. The 2- way interactions (treatment diet x breed) did not ( $P>0.05$ ) affect any of the carcass yield parameters.

**Table 4.1** Effects of diet and breed interaction on carcass yield of Boschveld and Potchefstroom Koekoek chickens.

Breed	Treatment	Slaughter weight (g)	Dressing weight (g)	Dressing %	Drumstick (g)	Breast muscle (g)	Thigh (g)	Wing (g)
BV	0% SP	993.00	571.68	57.33	102.04	130.64	104.50	84.63
	5% SP	966.67	649.00	66.54	91.65	132.97	93.26	78.94
	10% SP	918.33	644.68	69.01	82.95	87.03	74.75	69.00
	15% SP	1018.33	593.33	57.48	87.81	104.96	87.34	73.75
PK	0% SP	1025.68	705.00	68.67	111.37	132.74	104.42	90.60
	5% SP	1123.33	664.00	57.93	104.19	122.22	104.55	82.78
	10% SP	991.33	446.00	46.73	84.49	100.96	71.27	72.48
	15% SP	1034.33	505.00	49.83	104.08	97.02	88.55	77.14
SEM		133.44	104.71	6.31	10.00	12.66	8.88	5.92
P-value		0.95	0.45	0.11	0.90	0.76	0.86	0.10

The results of carcass cuts yield as influenced by the main effect of diet and breed are presented in Table 4.2. The breed did not ( $P>0.05$ ) influence the slaughter weight,

dressing weight and dressing percentage, and all the carcass cuts yield parameters. However, the dietary treatment had a significant ( $P < 0.05$ ) effect on the thigh and breast muscle weight, but did not ( $P > 0.05$ ) affect the slaughter weight, dressing weight, dressing percentage, drumstick and wing weights.

The results also indicate that, chickens fed diet 0% SP had heavy thigh weight (104.46g) which was similar ( $P > 0.05$ ) to that of chickens fed diet 5% SP and 15% SP. Chickens fed the diet with 10% SP and 15% SP showed a similar ( $P > 0.05$ ) thigh weights. The lowest ( $P < 0.05$ ) thigh weight was recorded in chickens fed diet 10% SP with 73.01g. Greater breast muscle weight was recorded in chickens fed diet 0% SP (131.69g) and diet 5% SP (127.60g), with the lowest ( $P < 0.05$ ) breast muscle weight observed in chickens offered diet 15% SP and 10% SP with values of 100.99g and 94.00g, respectively. However, diets 5% SP and 15% SP had similar ( $P > 0.05$ ) breast muscle weight.

**Table 4.2** Main effects of diet and breed on the carcass yield of two chicken breeds

<b>Diet</b>	<b>Slaughter weight (g)</b>	<b>Dressing weight (g)</b>	<b>Dressing Percentage (%)</b>	<b>Drumstick (g)</b>	<b>Breast Muscle (g)</b>	<b>Thigh (g)</b>	<b>Wing (g)</b>
<b>0% SP</b>	1009.33	638.33	63.00	106.70	131.69 <sup>a</sup>	104.46 <sup>a</sup>	87.61
<b>5% SP</b>	1045.00	656.50	62.23	97.92	127.60 <sup>ab</sup>	98.91 <sup>a</sup>	80.86
<b>10% SP</b>	955.33	545.33	57.87	83.72	94.00 <sup>c</sup>	73.01 <sup>b</sup>	70.74
<b>15% SP</b>	1026.33	549.17	53.66	95.95	100.99 <sup>bc</sup>	87.95 <sup>ab</sup>	75.44
<b>SEM</b>	94.42	74.04	4.46	7.07	9.00	6.28	4.19
<b>p-value</b>	0.92	0.61	0.44	0.19	0.02	0.01	0.06
<b>Breed</b>							
<b>BV</b>	974.33	614.67	62.59	91.11	113.90	89.96	76.58
<b>PK</b>	1043.50	580.00	55.79	101.03	113.24	92.20	80.75
<b>SEM</b>	65.72	52.39	3.15	5.00	6.33	4.44	2.96
<b>P-value</b>	0.47	0.65	0.15	0.18	0.94	0.73	0.33

<sup>abc</sup>Means in the same column with different superscripts are significantly different (P<0.05)

#### 4.4.2. Internal organs sizes

The results for 2- way interactions between the two chicken breeds (Boschveld and Potchefstroom Koekoek) and dietary treatments (0% SP, 5% SP, 10% SP and 15% SP) and their effects on internal organs weights are presented in Table 4.3. The 2-way interactions of breed x dietary treatment did not (P>0.05) affect the gizzard, spleen, heart, liver and intestines weights.

**Table 4.3** Internal organ weights of Boschveld and Potchefstroom Koekoek chickens fed four grower diets with different inclusion levels of *Spirulina platensis*.

<b>Breed</b>	<b>Diet</b>	<b>Gizzard</b>	<b>Spleen</b>	<b>Heart</b>	<b>Liver</b>	<b>Intestines</b>
BV	0% SP	28.64	1.63	5.84	14.82	41.24
	5% SP	26.10	2.39	4.79	18.80	53.22
	10% SP	20.79	1.26	5.91	17.49	38.08
	15% SP	20.94	2.01	4.60	16.95	45.10
PK	0% SP	34.36	2.75	6.06	19.21	51.78
	5% SP	27.28	2.41	5.65	19.81	45.93
	10% SP	26.86	1.96	4.41	15.03	29.78
	15% SP	28.02	2.10	5.32	16.71	38.15
SEM		4.33	0.32	0.44	1.60	6.07
P-value		0.91	0.30	0.06	0.23	0.37

The results on the influence of chicken breeds and dietary treatment on internal organs weights are presented in Table 4.4. The inclusion of Spirulina as a replacement of Soybean at different inclusion levels (0%, 5%, 10% and 15%) had no effect ( $P>0.05$ ) on the gizzard, spleen, heart, liver and intestine weights. Similarly, the chicken breed did also not ( $P>0.05$ ) influence the gizzard, heart, liver and intestine weights but affected ( $P<0.05$ ) the spleen weight. The Potchefstroom Koekoek had a heavier ( $P<0.05$ ) spleen (2.30g) as compared to the Boschveld (1.82g).

**Table 4.4** The main effects of the diets and breeds on internal organ sizes as influenced by inclusion of Spirulina in grower diet.

<b>Diet</b>	<b>Gizzard</b>	<b>Spleen</b>	<b>Heart</b>	<b>Liver</b>	<b>Intestines</b>
<b>0% SP</b>	31.50	2.19	5.95	17.01	46.51
<b>5% SP</b>	26.69	2.40	5.22	19.30	49.58
<b>10% SP</b>	23.83	1.61	5.16	16.26	33.93
<b>15% SP</b>	24.48	2.05	4.96	16.83	41.62
<b>SEM</b>	3.06	0.23	0.31	1.13	4.29
<b>p-value</b>	0.31	0.13	0.17	0.28	0.09
<b>Breed</b>					
<b>BV</b>	24.12	1.82 <sup>b</sup>	5.29	17.01	44.41
<b>PK</b>	29.13	2.30 <sup>a</sup>	5.36	17.69	41.41
<b>SEM</b>	2.17	0.16	0.22	0.80	3.04
<b>P-value</b>	0.12	0.05	0.82	0.56	0.50

<sup>ab</sup>Means within the same column with different superscripts differ significantly ( $P \leq 0.05$ )

## **4.5. Discussion**

### **4.5.1. Carcass yield**

Carcass yield is a primary concern for every chicken producer. The present study shows that the treatment diets did not affect slaughter weights, dressing weight and dressing percentage. These findings agree with those of Zahir *et al.* (2019) who reported a non-significant difference in dressing percentage and final live weight of chickens fed Spirulina diets. However, the results contradict those of Ansari *et al.* (2018) & Moustafa *et al.* (2021) who reported a significant increase in dressing percentage across the treatment diets when Spirulina was used in chicken diets. The dressing percentage and dressing weight may vary due to some of the factors such as difference in slaughter weights and different chicken breeds used in the experiments. Heavier breeds tend to have heavy slaughter weight which results in high dressing percentage or carcass yield (Omojola *et al.*, 2004). In the present study, there were no significant differences observed in slaughter weights, similarly slaughter weights did not differ among the breeds, hence there was no significant difference observed in dressing weight and dressing percentage. Meaty birds are also known to have high dressing percentage than dual purpose breeds (Deng *et al.*, 2022) which could have also contributed to differences in results among the studies done.

The present study recorded a non-significant difference in drumstick weight which is in agreement with findings of Ansari *et al.* (2018) who reported a non-significant difference in drumstick weight of broilers. However, the findings of the current study

contradict those of Mullenix *et al.* (2022) who reported a significant decrease in drumstick, wing and thigh weights of broilers fed standard corn/soybean diet (control) than those fed diet containing 10% Spirulina. The wing weights in the current study were not significant and this is in agreement with that of Mullenix *et al.* (2022) who reported no significant difference in wing weights of broilers in the diets containing Spirulina this could be attributed to no significant differences in slaughter weights in the current study.

This study demonstrated that dietary treatments significantly influenced breast muscle weight of both Potchefstroom Koekoek and Boschveld chickens. Heavy breast muscle was recorded in control group (0% SP) than in birds fed Spirulina diets at all level of inclusions.

These results are in agreement with those of Mullenix *et al.* (2022) who reported high breast yield in control group than in broilers fed 10% Spirulina diets. Similarly, the results of the study are also in support with the findings of Moustafa *et al.* (2021) who recorded a significant difference in breast muscle in broilers fed a 1% Spirulina diet yielding high breast weight than those on control diet. However, the results contradict those of Ansari *et al.* (2018) who recorded no significant difference in breast percentage of broiler chickens fed different levels of Spirulina. In the present study, treatment diets also influenced weights of the chicken`s thigh. All chickens on control diet had heavy thighs than those on Spirulina diets. The results are in agreement with that of Mullenix *et al.* (2022) who recorded a significant increase in thigh weights of broiler fed control diet than those fed Spirulina diets. The differences in breast muscle and thigh weights in

this study could be attributed to the significance in body weights as breast and thigh weight are highly correlated to body weight (Zhao *et al.*, 2007; Mullenix *et al.*, 2022)

The breed of chickens did not have an influence on the breast muscle and thigh weights in the present study. Although not considered in this study, gender may have also influenced breast and thigh weights, as there is a sex hormone believed to influence muscle development as males have a sex hormone that is thought to enhance muscle development more than the estrogen in females that is mostly for fat deposition than muscle development (Sebola *et al.*, 2015).

#### **4.5.2. Internal organ sizes**

There was no significant difference recorded among the treatment groups in the weight of gizzard, heart, spleen, intestine and liver. These results are in agreement with those of Zahir *et al.* (2019) who reported no significant difference in liver, gizzard, intestines, heart and liver of broiler in all treatments groups. The results also agree with that of Kaoud (2012) who reported a non-significant difference in gizzard weights but observed a decrease in liver weights in Spirulina supplemented groups. In the current study, the breed did not affect gizzard, liver, heart and intestine but significantly affected spleen weight. Potchefstroom Koekoek is a heavy breed than Boschveld, which explains the heavier spleen in Potchefstroom Koekoek than in Boschveld and this confirms that the spleen weight is in direct proportion to body size of an animal (Vali *et al.*, 2023).

#### **4.6. Conclusion and recommendations**

In conclusion, the present study indicated replacing soybean with Spirulina at different levels shows did not affect slaughter weights, dressing weight, dressing percentage and

wings 'weight and internal organ sizes. Breast muscle and thigh weights from chicken fed 0%SP and 5%SP was high. This indicates that inclusion of *Spirulina platensis* in chicken diets up to 5% per 100kg of feed does not negatively affect the carcass cuts yield. The study recommends that for further studies, the gender of the chickens be considered and be evaluated differently at each inclusion level for carcass cuts yield and internal organ sizes, this is because sex may influence the carcass cuts yield due to the difference in rate of muscle development in males and females birds.

#### 4.7. References

- Ansari, M. S, Hajati, H., Gholizadeh, F., Soltani, N.,& Alavi, M. (2018). Effect of different levels of spirulina platensis on growth performance, intestinal morphology, gut microflora, carcass characteristics and some blood parameters in broiler chickens. *Journal of Phycological Research*, 2(2): 187-197.
- Cabrol, M. B., Glisic, M., Almeida, A. M., Baltic, Z. M., Raymundo, A., & Lordelo, M. M. (2021). The effect of Spirulina inclusion in broiler feed on meat quality: Recent trends in sustainable production. *IOP Conf. Earth and Environmental Science*, 825: 012011.
- Deng, S; Xing, T; Li, C; Xu, X; Xhou, G. (2022). The effect of breed and age on the growth performance, carcass traits and metabolic profile in breast muscle of Chinese indigenous chickens. *Foods*, 11: 483.
- Department of Agriculture, Forestry & Fisheries (DAFF). (2006). Meat Safety Act (Act no. 20 of 2000, R153/2006). South African Government Gazette. 28520: 20-22.  
<https://www.nda.agric.za/vetweb/Legislation/Meat%20safety/Poultry%20Regulations.pdf>
- Hafeni, S., Mpofo, I. D. T., & Petrus, P. (2013). The potential of pearl millet and water melon seeds as cheap alternative ingredients in Namibian poultry feeds. *Agricultural Science Research Journal*, 3(5): 140-143.

- Kaoud, H. A. (2012). Effect of spirulina platensis as a dietary supplement on broiler performance in comparison with prebiotics. *Scientific Journal of Applied Research*, 2(2): 46–51.
- Kovač, D., Simeunović, J., Babić, O., Mišan, A. Č., & Milovanović, I. L. (2013). Algae in food and feed. *Food and Feed Research*, 40(1): 21-31.
- Masaire, E., Madzingira, O., Samkange, A., Kandiwa, E., Mushonga, B., & As, B. (2018). Characterization of poultry production and management systems in the communal areas of Namibia. *African Journal of Poultry Farming*, 6(3): 265–276.
- Mast, M. G., Th. Uijttenboogaart, G., Gerrits, A. R., & Devries, A. W. (1982). Effect of Auger- and Press-Type Mechanical Deboning Machines on Selected Characteristics of Mechanically Deboned Poultry. *Journal of Food Science*, 47(6): 1757-1763.
- Mirzaie, S., Zirak-khattab, F., Hosseini, S. A., & Donyaie-darian, H. (2018). Effects of dietary *Spirulina* on antioxidant status , lipid profile , immune response and performance characteristics of broiler chickens reared under high ambient temperature. *Asian-Australasian Journal of Animal Sciences*, 31(4): 556–563.
- Moustafa, E.S.; Alsanie, W.F.; Gaber, A.; Kamel, N.N.; Alaqil, A.A.; Abbas, A.O. (2021). Blue-Green Algae (*Spirulina platensis*) Alleviates the Negative Impact of Heat Stress on Broiler Production Performance and Redox Status. *Animals*, 11 (1243):1-13.
- Mullenix, G. T., Maynard, C. J., Owens, C. M., Rochell, S. J., Bottje, W. G., Brister, R.

- D., & Kidd, M. T. (2022). Spirulina platensis meal inclusion effects on broilers fed a reduced protein diet. *Journal of Applied Poultry Research*, 31: 100199.
- Omojola, A. B., Adeshinwa, A. O. K., Madu, H., & Attah, S. (2004). Effect of sex and slaughter weight on broiler chicken carcass. *Journal of Food, Agriculture & Environment*, 2:61-63.
- Packard, R. (2014). *Comparison of production parameters and meat quality characteristics of South African indigenous chickens* (Doctoral dissertation, Stellenbosch: Stellenbosch University)
- Park, J. H., Lee, S. I., & Kim, I. H. (2018). Effect of dietary Spirulina (Arthrospira) platensis on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission and breast meat quality of broiler chickens. *Journal of Poultry Science*, 97:2451-2459.
- Pestana, J.M., Puerta, B., Santos, H., Madeira, M. S., Lopes, P. A., Pinto, R. M. A., Lemos, J. P. C., Fontes, C. M. C. A., Lordelo, M. M., & Prates, J. A. M. (2020). Impact of dietary incorporation of Spirulina (Arthrospira platensis) and exogenous enzyme on broiler performance, carcass traits and meat quality. *Poultry Science*, 99(5): 1-14.
- Sebola, N. A., Mlambo, V., Mokoboki, H.K., & Muchenje, V. (2015). Growth performance and carcass characteristics of three chicken strains in response to incremental levels of dietary Moringa oleifera leaf meal. *Journal of Livestock Science*, 178:202-208.

- Singh, V.P., & Pathak, V. (2016). Contribution of carcass cuts in meat production of Kadaknath, Aseel and Vanraja breeds of chicken. *Journal of Animal Research*, 7(1):213-217.
- Uushona, T. (2015). *Black soldier fly (Hermetia illucens) pre-pupae as a protein source for broiler production* (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Vali, Y., Gumpenberger, M., Konicek, C., & Bagheri, S. (2023). Computed tomography of the spleen in chickens. *Frontiers in Veterinary Science*, 10, 1153582. <https://doi.org/10.3389%2Ffvets.2023.1153582>
- Venkataraman, L.V., T. Somasekaran, and E.W. Becker. 1994. Replacement value of blue-green alga *Spirulina platensis* for fishmeal and a vitamin mineral premix for broiler chicks. *British Poultry Science*, 35: 373–381.
- Zahir, U. R., Md. Anwarul, H. B., Maksuda, B., & Noushin, A. (2019). Effects of dietary supplement of dried Chlorella powder as an alternative to antibiotic on growth performance and health status of broiler chicken. *International Journal of Biosciences*. 14 (4): 255-263.
- Zhao, G. P., Chen, J. L., Zheng, M. Q., Wen, J., & Zhang, Y. (2007). Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. *Poultry Science*, 86(11): 2309-2314.

## CHAPTER 5

### EFFECTS OF FEEDING INCREMENTAL LEVELS OF *SPIRULINA PLATENSIS* ON INTESTINAL GUT MICROFLORA OF BOSCHVELD AND POTCHEFSTROOM KOEKOEK CHICKEN BREEDS

#### Abstract

A 12 weeks feeding trial was conducted using 72 unsexed chickens from the two chicken breeds, divided equally among the breeds. This study aimed at determining the effect of different inclusion levels of *Spirulina Platensis* on gut microflora of Boschveld and Potchefstroom Koekoek chicken breeds. The birds were fed with commercial starter crumbles from day 1 to week 4. At week 5 of age, the chickens were allocated randomly to four dietary treatments, which were as follows; 0% SP, 5% SP, 10% SP and 15% SP of dried *Spirulina platensis* powder as a replacement of soybean meal. The birds were fed the grower experimental diets for 12 weeks. A total of 24 birds were slaughtered at the end of the experiment and the cecal contents of the slaughtered birds were collected aseptically for microbial enumeration of bacteria. Data were analyzed using analysis of variance procedures based on general linear models of factorial experiments. The results on *Staphylococcus*, coliforms and *Lactobacillus* counts revealed no significant difference ( $P>0.05$ ) between the two chicken breeds. Similarly, the results indicated no significant interaction between the breed and dietary treatment on the *Staphylococcus*, coliforms and *Lactobacillus* counts. However, a significant decrease ( $P<0.05$ ) was observed in coliform counts with increase in Spirulina in the diet, while *Lactobacillus*

significantly increased with increase in Spirulina in the diet. In conclusion, the inclusion of *Spirulina platensis* in the grower diet of chickens have demonstrated a favorable effect on the cecal microbial loads of chickens in the current study by reducing the pathogenic bacteria coliform and increasing the beneficial *Lactobacillus* counts.

**Key words:** diet, breed, bacterial, counts,

## 5.1. Introduction

Gut flora is defined as microorganisms that exist in the digestive tract of an animal and are capable for a number of important functions for these animals (Adil & Magray, 2012). The digestive tract of poultry consists of unicellular microorganisms mainly bacteria, fungi and protozoans. The growth of microorganisms in the poultry gut involves complex processes that depend on numerous factors, starting from the age of the chicken to the feed ingredients used and the environment in which the chickens are raised (Yasothai, 2017). However, gut microflora plays a role in digestion and absorption of nutrients, removal of toxins from certain compounds, growth performance and protection of animals against invading pathogens (Amit-Romach *et al.*, 2004).

The interaction of the gut microbiota with the lining of the gastrointestinal tract may change the physiological structure of the tract and eventually affects the immune status of the animal (Van Leeuwen *et al.*, 2004). Also the gut microbiota provides a wide range of enzymes and substrates that provides an extensive metabolome that can be used for the derivation of nutrient and energy from the feed (Stanley *et al.*, 2013). Apparent metabolizable energy of the feed is related to the composition of the feed which is a result of the conversion of certain feed components into high energy metabolites by specific bacteria. The use of these fermented end products such as lactic acid can give sufficient energy to the host (Lan *et al.*, 2005) and finally results in good feed conversion ratio (Rinttilä & Apajalahti, 2013).

A healthy normal flora is the first line of defense for all animal`s bodies to pathogenic bacteria (Hofacre *et al.*, 2001). Understanding bacteria present in the chicken gut may allow one to understand how antibiotic growth promoters works and may also help with determining methods that can be used to achieve maximum growth performance and low feed efficiency without using antibiotics to manipulate the intestinal flora (Engberg *et al.*, 2000).

Spirulina is a nutrient dense source of food, with different nutrient components, antioxidants, and probiotics properties (Ansari *et al.*, 2018). Packard (2014) defined probiotics as specific microorganisms or groups of microorganisms, that have beneficial effect to the host by improving the structure of intestinal microflora. According to Yadav *et al.* (2016) existing probiotics can be categorized into colonizing species (*Lactobacillus sp.*, *Enterococcus sp.* and *Streptococcus sp.*) and free, non-colonizing species (*Bacillus sp.* and *Saccharomyces cerevisiae*). Probiotics inhibit bacterial growth by secreting products that inhibit the bacterial development. The products secreted are such as bacteriocins, organic acids and hydrogen peroxide (Packard, 2014). Patterson and Burkholder (2003) also highlighted that, probiotics compete with pathogens in terms of locations in order to attach to the intestinal mucous membranes which prevents pathogen bacteria from residing in the digestive tract. In doing this, they create an environment in the intestines which is favorable for the growth of useful bacteria and in a way inhibit the development of pathogenic bacteria. Therefore, the aim of this study was to investigate the effect of feeding graded levels of *Spirulina platensis* on the intestinal gut microflora of Boschveld and Potchefstroom Koekoek chickens.

## **5.2. Materials and Methods**

### **5.2.1 Experimental design, bird management and diets**

The materials and methods on the bird management and care, experimental design, treatment diet formulation and dietary treatment composition are outlined under Chapter 3 under sections 3.2.1, 3.2.2, 3.2.3 and 3.2.4, respectively.

### **5.2.2 Slaughtering process**

The details and procedure on the slaughtering process are outlined in Chapter 4 under section 4.2.2.

### **5.2.3. Sample collection**

At the end of the experiment, 24 birds were slaughtered and the cecal contents were collected from the cecum aseptically. The cecal content was collected in a sterile tube and taken to the laboratory. In the laboratory, one gram of cecal contents was transferred into a sterile dilution tube, containing 9 ml sterile nutrient broth as preparation for enumeration of bacteria following the procedure highlighted by Yusuf *et al.* (2016).

### **5.2.4. Microbiological analysis of bird samples**

#### **5.2.4.1. Enumeration of bacteria**

Standard plating method was used for microbial analysis of cecal contents from the chickens. Serial dilutions ( $10^1 - 10^{-3}$ ) were prepared from which 10  $\mu$ l of mixed diluted samples was mixed using a vortex and was plated using a pour plate technique described by Sanders (2012) on to the following media; MacConkey agar (Oxoid Ltd., United Kingdom) for the enumeration of *coliforms*, De Man-Rogosa- sharpe (MRS) agar

(Oxoid Ltd., England) for the enumeration of *Lactobacillus* and on Mannitol salt agar (Oxoid Ltd., United Kingdom) for the enumeration of total *Staphylococcus*.

The MacConkey agar and MRS plates were inverted and incubated at 37°C for 24 - 48h. While the Mannitol salt agar plates were incubated in 3% CO<sub>2</sub> atmosphere at 37°C for 48-72 h. After incubation, the plates were observed for bacterial growth and the counting was done by holding the plate to a light source and counting all colonies by marking their position on the back of the petri plates with a marking pen. Colonies between 30 and 300 were considered; colonies above 300 were not considered and recorded as TNTC (Too numerous to count), and those below 30 were recorded as TLTC (Too little to count) following a procedure by Sanders (2012). The results were recorded per media and per dilution. Each treatment per breed was represented three times during the experiment and the results were expressed as arithmetical means  $\pm$  SE (in log<sub>10</sub> cfu/g).

#### **5.2.4.2. Isolation and bacterial identification**

A sterile wire loop was used to pick up bacteria and the wire was moved lightly along the agar surface, depositing streaks of bacteria on the surface. The loop was flamed after each streaking. Thereafter, bacteria identification was performed for confirmation of the type of bacteria grown in the plates and a gram stain protocol by Smith & Hussey (2005) was followed. The isolates from different colonies of each bacterium were cultured with a sterile wire loop and emulsified in a small drop of sterile saline solution on a clean glass of microscope slide and spread evenly. All smears were allowed to dry and heat-fixed by-passing the slide over a Bunsen burner flame and allowed to cool before

staining. The fixed slide was flooded with Gentian violet stain, allowed to stain for minute and rinsed with water, followed by iodine solution for one minute, and working decolorizing solution for 10-30 seconds and lastly Safranin counterstain for one minute. The slide was then blotted and allowed to air dry before being examined under the microscope. From the microscope, bacteria on the slides were captured and identified according to their shapes and color; this was used as to confirm the type of bacteria grown on the plates.

### **5.2.5. Statistical analyses**

Data of this study were analyzed using analysis of variance (ANOVA) procedures based on general linear models of factorial experiments. Two-way ANOVA procedures were used to account for the effect of both the main and interaction for a 4 (diets) x 2 (chicken strains). Means separation and pairwise comparisons were done by Duncan's post hoc test. Statistical analyses were conducted by SPSS software (SPSS version 28). The linear model employed was as follows:

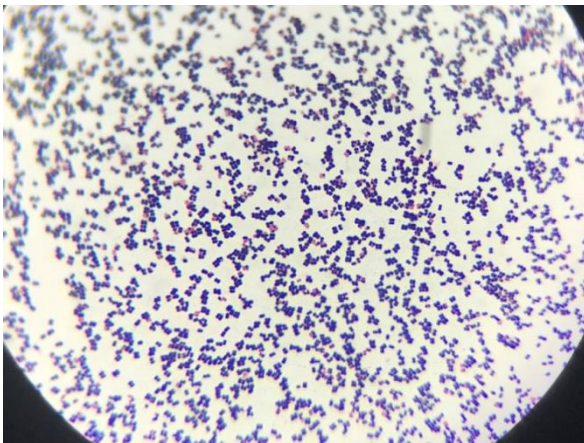
$$Y_{ijl} = \mu + D_i + B_j + (D \times B)_{ij} + E_{ij}$$

Where  $Y_{ijl}$  is the observation of the treatment of the dependent variable  $ij$ ,  $\mu$  the fixed effect of population mean variable,  $D_i$  = the effect of  $i^{\text{th}}$  treatment diet (0% SP, 5% SP, 10% SP, 15% SP);  $B_j$  = the effect of  $j^{\text{th}}$  chicken breed (BV, PK);  $(D \times B)_{ij}$  = the interaction effect of the  $i^{\text{th}}$  diet and  $j^{\text{th}}$  chicken breed;  $E_{ij}$  = the random error. Significance level was set at  $P < 0.05$ . Means indicating significant differences were tested using Duncan's multiple range test of SPSS.

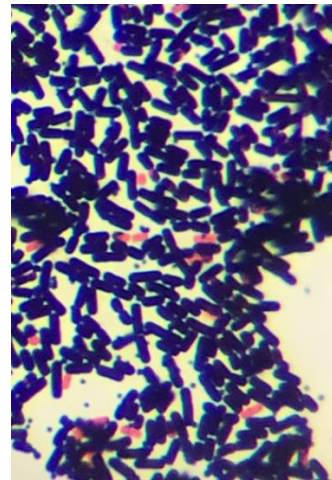
### 5.3. Results

#### 5.3.1. Bacteria identification

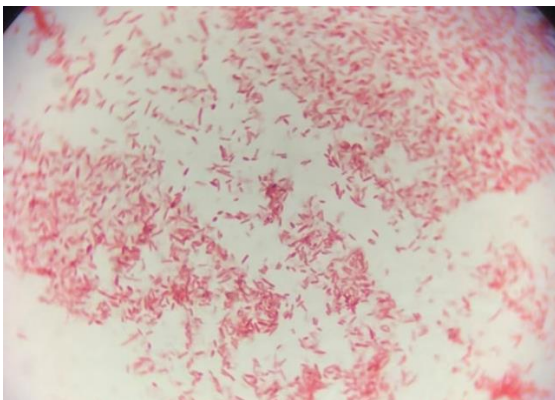
The results of the biochemical tests and that of gram-staining confirmed that the isolated bacteria were indeed the test bacteria. The images captured from the microscope are given in Figures 5.1-5.3 below.



**Figure 5.1** *Staphylococcus* bacteria



**Figure 5.2** *Lactobacillus* bacteria



**Figure 5.3** Coliforms

### 5.3.2. Enumeration of gut microflora

The findings on the two way interactions effect of breed x treatment on cecal microbial count is given in Table 5. 1. The study results indicate that breed x treatment diet did not affect ( $P>0.05$ ) the *Staphylococcus*, Coliforms and *Lactobacillus* counts.

**Table 5.1** Cecal microbial count (CFU/g) as influenced by the interaction of chicken breeds and treatment diets.

Breed	Diet	<i>Staphylococcus</i>	Coliforms	<i>Lactobacillus</i>
BV	0% SP	5.00	6.60	6.07
	5% SP	6.57	6.23	6.73
	10% SP	7.47	5.70	7.10
	15% SP	6.03	5.26	7.47
PK	0% SP	6.53	6.23	6.20
	5% SP	6.30	5.80	6.60
	10% SP	6.87	5.47	6.93
	15% SP	6.10	5.03	7.07
SEM		1.10	0.24	0.22
P-value		0.78	0.96	0.68

The results for the main effects of treatment diet and breed are presented in Table 5.2. The breed of chickens did not affect ( $P>0.05$ ) the microbial counts. Similarly, the results show that there was no significant difference ( $P>0.05$ ) in the *Staphylococcus* counts of chickens fed grower diet with different levels of Spirulina. However, treatment diets significantly influenced ( $P<0.05$ ) the *E. coli* counts and the *Lactobacillus* counts. Coliforms count was negatively affected by the inclusion of Spirulina in the diet, which

is indicated by a decrease in coliforms with an increase in Spirulina inclusion level. The *Lactobacillus* count increased in the chickens fed grower diet with Spirulina with the highest count recorded in chickens on diet 15% SP.

**Table 5.2** The main effects of diet and breed on cecal microbial count (cfu/g) of Potchefstroom Koekoek and Boschveld chickens.

<b>Diet</b>	<i>Staphylococcus</i>	<b>Coliforms</b>	<i>Lactobacillus</i>
0% SP	5.77	6.42 <sup>a</sup>	6.13 <sup>c</sup>
5% SP	6.43	6.02 <sup>ab</sup>	6.67 <sup>b</sup>
10% SP	7.17	5.58 <sup>b</sup>	7.02 <sup>ab</sup>
15% SP	6.07	5.15 <sup>c</sup>	7.27 <sup>a</sup>
<b>SEM</b>	0.78	0.17	0.15
<b>p-value</b>	0.62	0.001	0.001
<b>Breed</b>			
BV	6.27	5.95	6.84
PK	6.45	5.63	6.7
<b>SEM</b>	0.55	0.12	0.11
<b>P-value</b>	0.82	0.08	0.37

<sup>abc</sup> Means within the same column with the different superscripts differs (P<0.05)

#### 5.4. Discussion

A healthy normal flora is the first line of defense for all animal bodies to invading pathogens (Hofacre *et al.*, 2001). While that is true, it is reported that the health and performance of poultry is influenced by gastrointestinal normal flora (Yusuf *et al.*, 2016). Additionally, the cecum plays an important role in preventing colonization of pathogens and it helps with the synthesis and absorbability of dietary vitamins and additional nutrients (Park *et al.*, 2018). *Staphylococcus* is found to be one of the common inhabitants of the chicken intestinal tract (Syed *et al.*, 2020). The results from the current study show no significant difference in the *Staphylococcus* counts of the cecal content of the chickens fed diets with different Spirulina levels. The results agree with those of Yusuf *et al.* (2016) who recorded no significant difference in the *Staphylococcus* of Japanese quails fed two different diets with different Spirulina inclusion levels.

The study results also show a significant decrease in the coliform counts and increase in *Lactobacillus* counts within the different treatment diets. This could be attributed to the high phenolic compounds reported in the diets containing Spirulina which makes Spirulina a good antioxidant and good prebiotic. It is reported that feeding Spirulina containing diets may increase *Lactobacillus* population as Spirulina contains growth promoters in the form of unique polysaccharides that facilitates the growth of lactic acid bacteria (Cheong *et al.*, 2022). *Lactobacillus* bacteria are important probiotics that are used to improve the intestinal health and growth performance of poultry by inhibiting pathogens and control immunity (Li *et al.*, 2018). The decrease in coliforms with an increase in Spirulina can also be associated with the antibacterial activities of Spirulina

against coliforms due to the known presence of active ingredients such as alkaloids and lipopolysaccharides (Park *et al.*, 2018).

These results are in agreement with those reported in literature (Shanmugapriya *et al.*, 2015; Yusuf *et al.*, 2016; Ansari *et al.*, 2018; Fathi *et al.*, 2018; Park *et al.*, 2018 &) where a significant decrease in coliform counts and an increase in *Lactobacillus* count were found with increase in Spirulina level in the diets.

### **5.5. Conclusion and recommendations**

Inclusion of Spirulina at different levels in the chickens' diet significantly affected the *Lactobacillus* and total coliforms in the intestinal gut, proving that apart from nutritional benefits, Spirulina has gut health benefits to chickens when they consume it. Spirulina contains good amount of phenolic compounds, which makes it a good prebiotic. The total coliform counts in the cecal content decreased significantly with increase in Spirulina level in the chicken diets which suggests that *Spirulina platensis* has more health benefits which is related to its known high antioxidants properties and phytonutrient potential. Furthermore, *Lactobacillus* count was improved with inclusion of Spirulina in the grower diet of Potchefstroom Koekoek and Boschveld chickens which proves the excellent probiotic properties associated with Spirulina. In order to improve the intestinal health for chickens, this study recommends the inclusion of Spirulina in the chicken diets to promote the inhibition of pathogenic bacterial growth while promoting the growth of probiotic colonizing species. However, further studies are recommended to establish the exact amount of Spirulina that can be safely

incorporated in the chicken`s diets (starter, grower and finisher) that will improve both the feed intake, growth performance and gut microflora without compromising on one of the parameters.

## 5.6. References

- Adil, S., & Magray, S. N. (2012). Impact and manipulation of gut microflora in poultry: A review. *Journal of Animal and Veterinary Advances*, 11(6): 873–877.
- Amit-Romach, E., Sklan, D., & Uni, Z. (2004). Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. *Poultry Science*, 83: 1093-1098.
- Ansari, M. S, Hajati, H., Gholizadeh, F., Soltani, N.,& Alavi, M, (2018). Effect of different levels of spirulina platensis on growth performance, intestinal morphology, gut microflora, carcass characteristics and some blood parameters in broiler chickens. *Journal of Phycological Research*, 2(2): 187-197.
- Cheong, K. L., Yu, B., Chen, J., & Zhong, S. (2022). A comprehensive review of the cardioprotective effect of marine algae polysaccharide on the gut microbiota. *Foods*, 11(22): 3550.
- Department of Agriculture, Forestry & Fisheries (DAFF). 2006. Meat Safety Act (Act no. 20 of 2000, R153/2006). South African Government Gazette. 28520: 20-22. <https://www.nda.agric.za/vetweb/Legislation/Meat%20safety/Poultry%20Regulations.pdf>
- Engberg, R. M., Hedemann, M. S., Leser, T. D., & Jensen, B. B. (2000). Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. *Poultry science*, 79(9), 1311-1319.
- Fathi, M. A., Namra, M. M. M., Ragab, M. S., & Aly, M. M. M. (2018). Effect of dietary supplementation of algae meal (*Spirulina platensis*) as growth promoter on

- performance of broiler chickens. *Egypt Poultry Science*, 38 (ii): 375-389.
- Hofacre, C. L., White, D. G., Maurer, J. J., Morales, C., Lobsinger, C., & Hudson, C. (2001). Characterization of antibiotic-resistant bacteria in rendered animal products. *Avian Diseases*, 45: 953-961.
- Lan, Y., Versteegen, M. W. A., Tamminga, S., & Williams, B. A. (2005). The role of the commensal gut microbial community in broiler chickens. *World's Poultry Science Journal*, 61(1): 95-104.
- Li, Z., Wang, W., Liu, D., & Guo, Y. (2018). Effects of *Lactobacillus acidophilus* on the growth performance and intestinal health of broilers challenged with *Clostridium perfringens*. *Journal of Animal Science and Biotechnology*, 9(1): 1-10.
- Packard, R. (2014). *Comparison of production parameters and meat quality characteristics of South African indigenous chickens* (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Patterson, J. A., & Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science*, 82(4): 627-631.
- Park, J. H., Lee, S. I., & Kim, I. H. (2018). Effect of dietary *Spirulina* (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission and breast meat quality of broiler chickens. *Journal of Poultry Science*, 97:2451-2459.
- Rinttilä, T., & Apajalahti, J. (2013). Intestinal microbiota and metabolites-Implications for broiler chicken health and performance. *Journal of Applied Poultry Research*,

22: 647-658.

Sanders, E. R. (2012). Aseptic laboratory techniques: plating methods. *Journal of Visualized Experiments*, 63(e3064):1-18.

Shanmugapriya, B., Babu, S. S., Hariharan, T., Sivaneswaran, S., Anusha, M. B., & College, C. N. (2015). Dietary administration of spirulina platensis as probiotics on growth performance and histopathology in broiler chicks. *International Journal of Recent Scientific Research*, 6(2): 2650-2653.

Smith, A. C., & Hussey, M. A. (2005). Gram stain protocols. *American Society for Microbiology*, 1: 14.

Stanley, D., Geier, M. S., Denman, S. E., Haring, V. R., Crowley, T. M., Hughes, R. J., & Moore, R. J. (2013). Identification of chicken intestinal microbiota correlated with the efficiency of energy extraction from feed. *Veterinary Microbiology*, 164(1–2): 85–92.

Syed, M. A., Ullah, H., Tabassum, S., Fatima, B., Woodley, T. A., Ramadan, H., & Jackson, C. R. (2020). Staphylococci in poultry intestines: a comparison between farmed and household chickens. *Poultry Science*, 99(9): 4549-4557.

Van Leeuwen, P., Mouwen, J. M. V. M., Van Der Klis, J. D., & Verstegen, M. W. A. (2004). Morphology of the small intestinal mucosal surface of broilers in relation to age, diet formulation, small intestinal microflora and performance. *British Poultry Science*, 45(1): 41-48.

Yadav, A. S., Kolluri, G., Gopi, M., Karthik, K., & Singh, Y. (2016). Exploring

alternatives to antibiotics as health promoting agents in poultry-a review. *Journal of Experimental Biology and Agricultural Sciences*, 4(3s): 368-383.

Yasothai, R. (2017). Importance of gut microflora in poultry. *International Journal of Science, Environment and Technology*, 6(1): 549–552.

Yusuf, M. S., Hassan, M. A., Abdel-Daim, M. M., El Nabtiti, A. S., Ahmed, A. M., Moawed, S. A., & Cui, H. (2016). Value added by *Spirulina platensis* in two different diets on growth performance, gut microbiota, and meat quality of Japanese quails. *Veterinary World*, 9(11): 1287.

**CHAPTER 6**  
**THE EFFECTS OF FEEDING INCREMENTAL LEVELS OF *SPIRULINA***  
***PLATENSIS* ON MEAT QUALITY AND SENSORY ATTRIBUTES OF**  
**BOSCHVELD AND POTCHEFSTROOM KOEKOEK CHICKEN BREEDS**

**Abstract**

The viability of a potential chicken feed depends on its effect on the quality of the end product which is meat or eggs. This study aimed at assessing the effect of incremental level of *Spirulina platensis* (SP) on meat quality and sensory attributes of Boschveld and Potchefstroom Koekoek chickens. A 16 weeks feeding trial was conducted using 72 unsexed chickens from the two chicken breeds, divided equally among the breeds. The birds were fed with commercial starter crumbles from day 1 to week 4. At week 5 of age, the chickens were randomly allocated to the 4 experimental diets. The experimental diets were as follows; 0% SP, 5% SP, 10% SP and 15% SP of dried *Spirulina platensis* powder as a replacement of soybean meal. The birds were fed the grower experimental diets for 12 weeks. A total of 24 birds were slaughtered at the end of the experiment. After 24 hours post mortem, the ultimate pH was measured from the breast samples and thereafter, the breast muscle samples were vacuum-packed and kept in the freezer at -18 °C until further analysis on meat proximate composition and sensory analyses. The data on proximate composition, pH, drip loss, cooking loss and sensory analyses were analyzed using the general linear models (GLM) procedure in SPSS for a two-way analysis of variance (ANOVA) for a 4 (diets) x 2 (chicken strains) factorial treatment arrangement. The interaction between diet and breed did not significantly ( $P>0.05$ )

affect the meat proximate composition. However, panelists discriminated some sensory attributes with dietary treatments by which diet significantly ( $P < 0.05$ ) influenced the flavor, initial juiciness and sustained juiciness of the chicken meat. Meat from control group (0%) had a prominent chicken flavor than meat from Spirulina fed groups (5%, 10% & 15%). Initial juiciness and sustained juiciness was more in meat from control group than meat from Spirulina groups. In conclusion, the inclusion of Spirulina in chicken grower diets does not affect chicken meat quality but influence the eating quality on some sensory attributes.

**Key words:** diet, breed, meat, quality, composition

## 6.1. Introduction

Chicken meat and its products are popularly consumed worldwide due to their distinctive characteristics of less fat and low amount of cholesterol (Choi *et al.*, 2016), and they are considered healthier than red meat by the modern consumers (Kralik *et al.*, 2018). Furthermore, the demand of chicken has increased in the past due to its low price and less religious limitations compared to other types of meat (Sebola *et al.*, 2015). Many households in rural areas raise indigenous chickens due to their adaptability (Masaire *et al.*, 2018) and low feed consumption (Van Marle-Koster & Webb, 2000). Indigenous chickens have a strong chicken flavor and texture (Petrus, 2011), which contributed to the growing demand for their meat in urban areas (Melesse, 2014). Nutritionally, chicken meat is a good source of protein in human diets (Marangoni *et al.*, 2015). However, there is a scarcity of scientific information on the flavor and texture characteristics of indigenous chickens.

Indigenous chickens are slow growing (Packard, 2014) and slow growth results in long rearing period which in the same way results in high costs of production as extra feed and extra health care is required over a long period. The performance of indigenous chickens can be improved by improving feeding among other many practices (Masaire, *et al.*, 2018). Scarcity of protein sources in chicken feeds has led poultry farmers and nutritionist to search for alternative protein sources such as micro algae (i.e., *Spirulina platensis*). *Spirulina* has a good nutritional profile, hence, feeding *Spirulina* containing diets may potentially enhance the absorbability of dietary vitamins, minerals, amino acids and other nutrients and promote growth (Mariey *et al.*, 2012).

Spirulina is mostly found in Africa, Asia and South America (Vonshak, 2002). Spirulina has high protein content 71% in dry matter; it contains antioxidants such as beta carotene and vitamin E which could improve the physico-chemical parameters of meat (Altmann *et al.*, 2018). The beta-carotene in algae and leafy green vegetables has high antioxidants effects than synthetics beta-carotene (Rubel, 2018). Spirulina has the ability to be cultivated in photo bioreactors and in natural ponds which could reduce the area required for cultivation (Taelman *et al.*, 2015).

Numerous studies have reported that supplementation of Spirulina affect meat quality, by which inclusion of Spirulina at 0.5 %, 1 % & 1.5 % in broiler diet have increased meat yellowness of broiler chickens (Moustafa *et al.*, 2021). Additionally, Spirulina was found to decrease the amount of water lost when meat is stored and reduce cooking loss of broiler meat (Altmann *et al.*, 2018). The production of indigenous chickens for bigger markets relies mainly on whether the consumer of the final products such as meat and eggs prefer its quality and eating quality (Packard, 2014). Therefore, the objective of this study was to determine the effect of *S. platensis* on meat quality and sensory attributes of Boschveld and Potchefstroom Koekoek chickens.

## **6.2. Materials and methods**

The materials and methods on the bird management and care, experimental design, treatment diet formulation and dietary treatment composition are outlined under Chapter 3 under section, 3.2.1, 3.2.2, 3.2.3 and 3.2.4 respectively.

### **6.2.1. pH measurement**

The carcass initial pH measurement was taken 15 min after slaughter from the right breast using a Testo 205 pH meter (Testo inc., 40 White Lake Rd, Sparta, USA) and thereafter, the carcass was chilled at 4 °C for 24 h. At 24 h the ultimate post-mortem pH was measured and recorded.

### **6.2.2. Thaw loss and cooking loss**

Thaw loss of chicken breast muscle was determined according to a method outlined by Honikel (1998) and cooking loss was determined using a modified method of Honikel (1998). The frozen breast samples were weighed prior to defrosting them at 4°C for ±12 hours, blot dried and weighed again. Thaw loss was determined for individual breasts muscle by calculating the weight differences between frozen and thawed breast samples. The breast muscle samples were placed in oven roasting bags and cooked in KIC oven at 160 °C for 30 minutes. Thereafter, the cooked samples were taken out of the oven when they reached an internal breast muscle temperature of 71°C; the temperature was measured using a thermometer. The cooked samples were cooled at room temperature for 15 minutes, blot dried and weighed. The weight after cooking and weight before cooking was used to calculate cooking loss as outlined by Honikel (1998) .

### **6.2.3. Proximate analyses**

The chickens breast samples were analyzed for dry matter, crude protein and ash according to the standard methods provided by the Association of Official Methods of Analysis (AOAC, 2016), as detailed in section 3.3.4. The crude fat content of the meat samples was determined according to AOAC (2016) method no.991.36 using petroleum ether as a solvent with a soxhlet extractor.

### **6.2.4. Sensory analysis**

A descriptive sensory analysis was performed on 24 breast cubes being the experimental units. The experimental design was: 4 diets x 2 chicken breeds x 3 replications. In this study, 6 meat science final year students were screened and trained on sensory characterization of chicken meat. The panel was trained for two days according to the research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of meat (AMSA, 1995). Thereafter, the panelist learned to identify the sensory attributes required for describing the overall aroma, flavor, sour taste, bitter taste, initial juiciness, sustained juiciness, tenderness and mealiness of chicken meat and was defined to be evaluated for descriptive sensory analysis.

The descriptive sensory evaluation was conducted using modified method of AMSA (1995). The samples were cooked as described for cooking loss in section 6.3.2 above and preserve in a thermal bag to maintain the temperature. The cooked breast samples were then cut into 1cm x 1cm pieces and wrapped in foil. The samples were warmed in a microwave for 30 seconds and immediately served while warm; each panelist evaluated four meat pieces per session, one for each treatment which was coded.

Descriptive sensory evaluation took place in two separate sessions. The panelists were provided with water and slices of apples for palate neutralization. A scale of 0 (none) to 10 (prominent) was used to evaluate the samples (AMSA, 1995). The scoring was performed on a pre-set hard copy questionnaire (Appendix D). The evaluation took place at the University of Namibia, department of Animal Production, Agribusiness and Economics boardroom. Prior to evaluation of the samples, the panelist voluntarily signed a consent form (Appendix C).

### 6.2.5. Statistical analyses

The meat quality parameters (pH at 15 minutes after slaughter, pH at 24 hours, thaw loss, cooking loss, proximate composition and sensory) data were analyzed using the general linear models (GLM) procedure in SPSS v28 (2020) for two-way analysis of variance (ANOVA) for a 4 (diets) x 2 (chicken strains) factorial treatment arrangement. The linear model employed was as follows:

$$Y_{ijkl} = \mu + D_i + B_j + P_k + (DB)_{ij} + (DP)_{ik} + (BP)_{jk} + (DBP)_{ijk} + E_{ijk}$$

Where  $Y_{ijkl}$  is the observation of the treatment of the dependent variable  $ij$ ,  $\mu$  = fixed effect of population mean variable,  $D_i$  = the effect of  $i^{\text{th}}$  treatment diet (0% SP, 5% SP, 10% SP, 15% SP);  $B_j$  = the effect of  $j^{\text{th}}$  chicken breed (BV, PK);  $P_k$  = the effect of panelist;  $(D \times B)_{ij}$  = the interaction effect of the  $i^{\text{th}}$  diet and  $j^{\text{th}}$  chicken breed;  $(D \times P)_{ik}$  = the interaction effect of the  $i^{\text{th}}$  diet and  $k^{\text{th}}$  panelist;  $(B \times P)_{jk}$  = the interaction effect of the  $j^{\text{th}}$  breed and  $k^{\text{th}}$  panelist;  $(D \times B \times P)_{ijk}$  = the interaction effect of the  $i^{\text{th}}$  diet,  $j^{\text{th}}$  chicken breed and  $k^{\text{th}}$  panelist;  $E_{ijk}$  = the random error. Significance level was set at  $P < 0.05$ . The significant means were compared using Duncan's post hoc test.

### 6.3. Results

#### 6.3.1. Meat quality

The mean values proximate composition (moisture, ash, crude fat and crude protein), thaw loss, cooking loss and pH<sub>u</sub> of meat as influenced by chicken breeds and dietary treatment are shown in Table 6.1. The two-way interactions of; diet x breed; diet x panelist, breed x panelist and 3 way interactions (diet x breed x panelist) did not (P>0.05) affect pH<sub>u</sub>, thaw loss, cooking loss and proximate composition of the chicken meat.

**Table 6.1** The effect of breed and dietary interaction on proximate composition, thaw loss, cooking loss and meat pH as influenced by incremental levels of Spirulina.

Breed	Diet	Moisture %	Ash %	Crude fat %	Crude protein %	Thaw loss %	Cooking loss %	pH <sub>u</sub>
<b>BV</b>	<b>0% SP</b>	75.86	1.33	8.16	25.92	8.18	19.25	6.23
	<b>5% SP</b>	74.80	1.14	7.08	24.90	6.99	20.99	5.76
	<b>10% SP</b>	75.79	1.10	6.70	24.45	11.58	22.67	6.33
	<b>15% SP</b>	75.52	1.11	6.11	25.07	8.53	20.00	6.28
<b>PK</b>	<b>0% SP</b>	75.15	1.07	6.09	25.50	6.61	22.98	5.92
	<b>5% SP</b>	74.90	1.08	6.97	25.73	11.34	22.08	6.01
	<b>10% SP</b>	75.83	1.07	6.84	24.10	13.52	25.48	6.11
	<b>15% SP</b>	74.83	1.09	6.72	23.20	8.54	25.99	6.05
<b>SEM</b>		0.71	0.07	0.67	0.79	1.94	2.67	0.20
<b>p-value</b>		0.90	0.24	0.24	0.43	0.47	0.83	0.36

pH<sub>u</sub>: Ultimate pH at 24 hours, BV= Boschveld, PK= Potchefstroom Koekoek

The mean values of meat pH<sub>u</sub>, thaw loss %, cooking loss % and proximate composition (moisture, ash, crude fat and crude protein) as influenced by the main factors (chicken breeds and dietary treatments) is shown in Table 6.2. Treatment diets did not significantly ( $P>0.05$ ) influence the moisture, ash, crude fat and crude protein contents of the meat. Similarly, chicken breeds did not affect ( $P>0.05$ ) proximate composition, pH, thaw loss and cooking loss of the chicken meat.

**Table 6.2.** The effect of diets and chicken breeds on proximate composition, thaw loss, cooking loss and meat pH.

Diets	Moisture %	Ash %	Crude fat %	Crude protein %	Thaw loss %	Cooking loss %	pH <sub>u</sub>
0% SP	75.50	1.20	7.13	25.71	7.40	21.11	6.08
5% SP	74.85	1.11	7.02	25.32	9.16	21.53	5.84
10% SP	75.81	1.07	6.77	24.26	12.67	24.08	6.22
15% SP	75.18	1.10	6.42	24.13	8.54	23.00	6.17
SEM	0.50	0.05	0.47	0.56	1.38	1.89	0.14
<i>P</i> -value	0.58	0.29	0.72	0.17	0.08	0.67	0.26
<b>Breed</b>							
BV	75.49	1.16	7.01	25.08	8.82	20.73	6.13
PK	75.18	1.08	6.67	24.63	10.06	24.13	6.02
SEM	0.35	0.03	0.33	0.40	0.97	1.33	0.10
<i>P</i> -value	0.537	0.110	0.457	0.434	0.380	0.090	0.466

pH<sub>u</sub>: Ultimate pH at 24 hours, BV= Boschveld, PK= Potchefstroom Koekoek

### 6.3.2. Descriptive sensory analysis and correlations

The statistical significance of the main treatments (diet, breed and panelist) and their interactions on the sensory attributes are given in Table 6.3. The two-way interactions of diet x breed and diet x panelist did not influence ( $P>0.05$ ) any of the sensory attributes. The two-way interaction of breed x panelist influenced ( $P<0.05$ ) the bitter taste of the meat.

**Table 6.3** Statistical significance of the effects of main factors (diet, breed and panelist) and their interactions on sensory attributes of chicken meat as influenced by incremental levels of Spirulina

Attributes	Effect of treatment			Interaction		
	Diet <sup>1</sup> (D)	Breed <sup>2</sup> (B)	Panelist (P)	D x B	D x P	B x P
Chicken aroma	NS	NS	NS	NS	NS	NS
Chicken flavor	*	NS	NS	NS	NS	NS
Sour taste	NS	NS	NS	NS	NS	NS
Bitter taste	NS	NS	NS	NS	NS	NS
Initial juiciness	*	NS	NS	NS	NS	NS
Sustained juiciness	*	NS	NS	NS	NS	NS
Tenderness	NS	NS	NS	NS	NS	NS
Mealiness	NS	NS	NS	NS	NS	NS
Acceptability	NS	NS	NS	NS	NS	NS

**Diet<sup>1</sup>** = 0% SP, 5% SP, 10% SP and 15% SP **Breed<sup>2</sup>** = Potchefstroom Koekoek and Boschveld, NS = Not significant ( $P>0.05$ ), \* = Significant ( $P <0.05$ )

The panelists determined that the dietary treatment significantly influenced ( $P<0.05$ ) the chicken flavor, initial juiciness and sustained juiciness of the meat as given in Table 6.4. The meat from 0% SP fed group was found to have a prominent chicken flavor compared to the meat from chicken fed Spirulina diets (5% SP, 10% SP and 15% SP). Furthermore, meat from chicken group fed 5% SP and 0% SP had more initial juiciness ( $P<0.05$ ) than meat from chickens fed 15% SP and 10% SP. Chicken meat from groups fed 0% SP, 5% SP and 15% SP recorded better sustained juiciness ( $P<0.05$ ) compared to meat from 10% SP group.

**Table 6.4.** Effect of incremental levels of *Spirulina platensis* on sensory attributes of two chicken breeds.

Attributes	Diets				SEM	P-value
	0% SP	5% SP	10% SP	15% SP		
Chicken aroma	7.33	5.67	5.17	5.00	0.594	0.056
Chicken flavor	7.25 <sup>a</sup>	6.33 <sup>ab</sup>	4.92 <sup>b</sup>	5.33 <sup>b</sup>	0.475	0.029
Sour taste	2.58	2.92	2.08	2.83	0.534	0.456
Bitter taste	2.42	2.33	2.42	2.42	1.449	0.998
Initial juiciness	5.25 <sup>a</sup>	5.75 <sup>a</sup>	3.42 <sup>b</sup>	4.33 <sup>ab</sup>	0.471	0.024
Sustained juiciness	5.75 <sup>a</sup>	6.17 <sup>a</sup>	3.5 <sup>b</sup>	5.00 <sup>a</sup>	0.487	0.009
Tenderness	6.25	5.42	4.83	5.25	0.519	0.323
Mealiness	6.17	6.17	4.75	4.92	0.599	0.106
Acceptability	6.00	5.67	3.67	4.67	0.499	0.057

<sup>ab</sup>Means in the same row with different superscripts are significantly different ( $P<0.05$ )

The results on correlations of the sensory attributes and proximate compositions are given in the Table 6.5. The correlations to be discussed for the purpose of this study are those in Table 6.6 below. Chicken overall aroma intensity was found to be highly correlated ( $r = 0.714$ ) to chicken flavor. Chicken aroma was also correlated moderately to acceptability of the meat ( $r = 0.501$ ). Initial juiciness was highly correlated ( $r = 0.880$ ) to sustained juiciness. Initial juiciness had a low correlation ( $0.398$ ) with crude fat content of the meat.

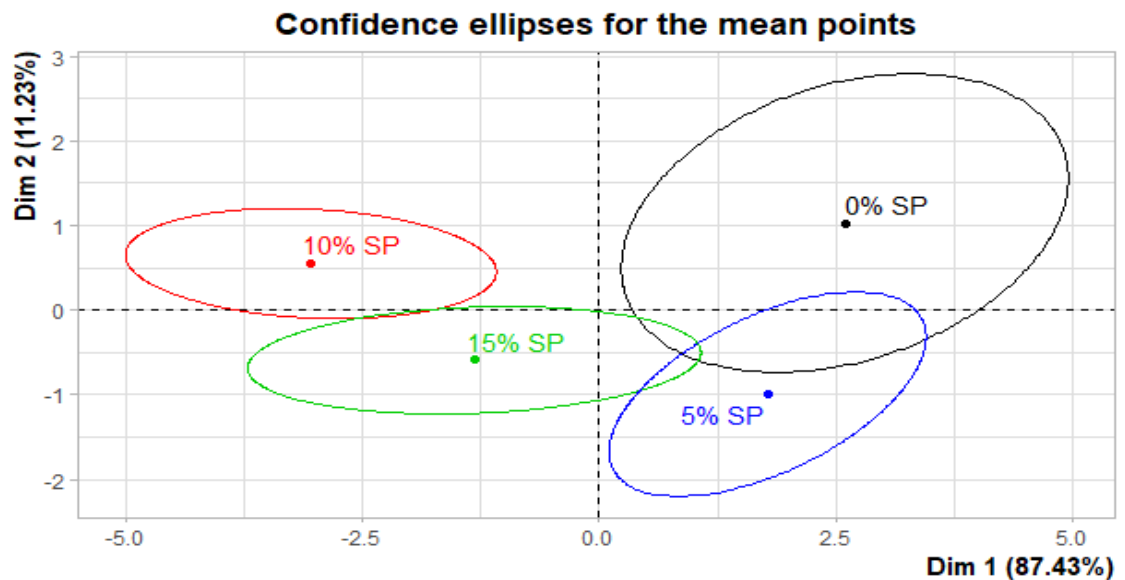
**Table 6.5** Correlation coefficients (r) and P values for meat samples as influenced by inclusion of *Spirulina platensis* in grower diet at different levels.

Attributes	1	2	3	4	5	6	7	8	9	10	11	12
1. Aroma intensity	1	0.714 0.001	0.211 0.323	0.257 0.225	0.207 0.331	0.192 0.369	0.29 0.17	0.308 0.143	0.505 0.012	0.309 0.142	0.095 0.66	0.175 0.413
2. Chicken flavor	0.714 0.001	1	0.052 0.809	0.117 0.586	0.432 0.035	0.299 0.155	0.282 0.183	0.453 0.026	0.568 0.004	0.316 0.132	0.131 0.543	0.163 0.446
3. Sour taste	0.211 0.323	0.052 0.809	1	0.416 0.043	0.437 0.033	0.48 0.018	0.131 0.541	0.42 0.041	0.36 0.084	-0.024 0.911	0.274 0.196	-1.2 0.576
4. Bitter taste	0.257 0.225	0.117 0.586	0.416 0.043	1	0.017 0.936	0.053 0.806	-0.067 0.756	0.025 0.907	0.088 0.682	0.273 0.197	0.121 0.573	0.051 0.812
5. Initial juiciness	0.207 0.331	0.432 0.035	0.437 0.033	0.017 0.936	1	0.88 0.001	0.362 0.083	0.653 0.001	0.751 0.001	0.021 0.923	-0.036 0.869	0.398 0.054
6. Sustained juiciness	0.192 0.369	0.299 0.155	0.48 0.018	0.053 0.806	0.88 0.001	1	0.366 0.078	0.609 0.002	0.774 0.001	-0.088 0.681	-0.028 0.898	0.276 0.191
7. Tenderness	0.29 0.17	0.282 0.183	0.131 0.541	-0.067 0.756	0.362 0.083	0.366 0.078	1	0.621 0.001	0.355 0.088	0.043 0.841	0.136 0.527	-0.11 0.61
8. Mealiness	0.308 0.143	0.453 0.026	0.42 0.041	0.025 0.907	0.653 0.001	0.609 0.002	0.621 0.001	1	0.644 0.001	-0.081 0.706	0.316 0.133	0.011 0.959
9. Acceptability	0.505 0.012	0.568 0.004	0.36 0.084	0.088 0.682	0.751 0.001	0.774 0.001	0.355 0.088	0.644 0.001	1	0.009 0.967	-0.03 0.89	0.254 0.231
10. Moisture %	0.309 0.142	0.316 0.132	-0.024 0.911	0.273 0.197	0.021 0.923	-0.088 0.681	0.043 0.841	-0.081 0.706	0.009 0.967	1	-0.283 0.181	0.229 0.283
11. Crude protein %	0.095 0.66	0.131 0.543	0.274 0.196	0.121 0.573	-0.036 0.869	-0.028 0.898	0.136 0.527	0.316 0.133	-0.03 0.89	-0.283 0.181	1	-0.285 0.176
12. Crude fat %	0.175 0.413	0.163 0.446	-0.12 0.576	0.051 0.812	0.398 0.054	0.276 0.191	-0.11 0.61	0.011 0.959	0.254 0.231	0.229 0.283	-0.285 0.176	1

**Table 6.6** Relevant positive correlation of sensory and chemical attributes of chicken breast muscle as influenced by inclusion of *Spirulina platensis* in grower diet of chickens

Parameters	Aroma intensity		Chicken flavor		Initial juiciness		Sustained juiciness		Acceptability		Crude fat	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
<b>Aroma intensity</b>			0.714	0.001					0.505	0.012		
<b>Chicken flavor</b>												
<b>Initial Juiciness</b>							0.88	0.001	0.751	0.001	0.398	0.054

The mean points data in Figure 6.1 show that, in overall, the sensory attributes for 0% SP, 5% SP & 15% SP do not differ significantly ( $P > 0.05$ ). However, 10% SP sensory attributes differs significantly ( $P < 0.05$ ) from 0% SP and 5% SP.



**Figure 6.1** Mean points for sensory attributes per treatment diets as influenced by incremental levels of Spirulina.

## 6.4. Discussion

### 6.4.1. Meat quality

The lack of effect of Spirulina on the ultimate pH in this study is in agreement with that of El-Bahr *et al.* (2020), Moustafa *et al.* (2021), Park *et al.*, 2018 and Pestana *et al.* (2020) who fed Spirulina to broilers. The lack of significant differences on meat pH may be attributed to the balanced nutritional composition of the diets that were iso-nitrogenous and iso-energetic that neither influenced meat fat. Lower carcass fatness is associated with higher final pH (Berri *et al.*, 2008) and low muscle glycogen increases meat pH (Raach-Moujahed *et al.*, 2011). Furthermore, stress ante-mortem is also known to negatively influence meat ultimate pH (Raach-Moujahed *et al.*, 2011). Therefore, the lack of differences observed in this study for meat muscle pH may be an indication that the birds were not exposed to stress prior to slaughter.

The current study, observed that thaw loss remained significantly unchanged in all treatment groups. The results are in agreement with findings of El-Bahr *et al.* (2020) who fed 1% Spirulina to broilers. The insignificant effect of Spirulina incremental level on cooking loss in this study is in accordance with results observed by Park *et al.* (2018) and Pestana *et al.* (2020). However, our results contradict those of El-Bahr *et al.* (2020) who reported Spirulina reduced cooking loss. The lack of significant difference in cooking loss among the treatment diets from the present study is attributed to lack of significant differences among the fat contents of the meat within the treatment diets as it is known that there is a significant positive correlation between lipid content and cooking loss (Watanabe *et al.*, 2018). However, other factors such as cooking method,

cooking temperature and cooking duration are also known to affect cooking loss of meat (Ježek *et al.*, 2020).

Regarding moisture, crude protein and crude fat content of the breast muscle no significant differences were observed with Spirulina inclusion, which agrees with the study of Altmann *et al.* (2018), who reported no differences in protein and moisture in broilers fed Spirulina. The results demonstrate that Spirulina inclusion in the chicken diets, yield similar results with control diet which uses soybean meal. In general, lack of differences on carcass proximate parameters is attributed to no noticeable differences found in the feed proximate parameters (Table 3.2), that lead to similar nutritional composition in the chicken meat (Pieterse *et al.*, 2019).

#### **6.4.2. Sensory analysis**

The inclusion of Spirulina in the grower diets of Boschveld and Potchefstroom Koekoek chickens resulted in a reduced chicken flavor in meat from chicken fed Spirulina diet than those fed control diet. This may be attributed to the strong oceanic scent of Spirulina powder in the diet (Rajmohan, 2017). The strong oceanic smell of Spirulina may be attributed to the presence of phenolic compounds which may have strongly influenced the flavor of the chicken meat from the Spirulina fed group. Chicken meat from 0% SP and 5% SP group were found to have more initial and sustained juiciness compared to chicken meat from 10% SP and 15% SP groups. The high juiciness in chicken meat from 0% SP and 5% SP may be related to high feed intake in the same group which translated in high chicken weight and increased muscle growth and subsequently increased intramuscular fat (Kandeepan, 2009) which is known to be

highly correlated to initial juiciness of the meat (Juárez *et al.*, 2011). The results from this study are in accordance with the findings of Pestana *et al.* (2020) who reported a significant reduction in juiciness of meat from chickens fed Spirulina and a non-significant difference on tenderness, flavor and overall acceptability of the chicken meat from the treatment groups. The results from the study are not in agreement with those of Raach-Moujahed *et al.* (2011), who found no significance difference in the sensory attributes across all treatments. Furthermore, this study results contradict the findings of Altmann *et al.* (2018), who reported that diet affected tenderness were by control group had more tender meat than Spirulina group. Differences in results not agreeing with others on sensory attributes are could be related to the evaluation method used for sensory analysis, the inclusion level of Spirulina used in the experiment, the dietary treatment composition and the method used for cooking the test samples.

Chicken flavor which was affected by diet was correlated to the overall meat acceptability, in which meat from chickens fed 10%SP and 15% SP had less chicken flavor and the acceptability was low compared to chicken meat from 0%SP and 5% SP groups. This study results show that initial juiciness correlated highly with sustained juiciness indicating that they provide similar juiciness attributes assessments in cooked chicken breast meat (Zhuang *et al.*, 2017). Fat percentage is expected to be positively related to tenderness (Uushona, 2015), although in this study no significant correlation was observed for fat and tenderness, and this likely due to similar observed meat fat content. However, crude fat was found to be correlated to initial juiciness of the meat from this study although the correlation was weak ( $r=0.398$ ). A no significant correlation to weak correlation between fat content of meat and initial juiciness is

expected as fat provide lubrication between muscle fibers and this increase the perception of juiciness ((Juárez *et al.*, 2011).

## **6.5. Conclusion and Recommendations**

It is anticipated that the viability of an alternative feed depends on its ability to affect the end product quality. The inclusion of *Spirulina platensis* in the grower diets of Boschveld and Potchefstroom Koekoek chickens did not influence the quality of the meat produced, which indicates that *Spirulina* can be incorporated in indigenous chicken diets without affecting the meat proximate composition. However, the flavor, initial juiciness and sustained juiciness were compromised when the level of *Spirulina* in the diets is increased above 5%, this suggests that there could be some anti-nutritional factors or phytochemicals present in *Spirulina* that have effect on the meat flavor and juiciness; this must be further investigated before including *Spirulina* at high level in the chicken feeds at a large scale. This study recommends further studies on the phytochemicals that could be present in *Spirulina* that affects the sensory attributes of the meat. It is also recommendable that further studies must consider the effect of gender on meat quality, this is important as gender can affect the meat flavor and acceptability of meat.

## 6.6. REFERENCES

- Altmann, B. A., Neumann, C., Velten, S., Liebert, F., & Mörlein, D. (2018). Meat quality derived from high inclusion of a micro-alga or insect meal as an alternative protein source in poultry diets: A pilot study. *Foods*, 7: 34.
- American Meat Science Association (AMSA). (1995). Research guidelines for cookery, sensory evaluation and instrumental measurements of fresh meat. American Meat Science Association in cooperation with the National Live Stock and Meat Board, Chicago, IL.
- Association of the Official Analytical Chemists (AOAC). (2016). Official Methods of Analysis of AOAC international. 20<sup>th</sup> Edition.
- Berri, C., Besnard, J., & Relandeau, C. (2008). Increasing dietary lysine increases final pH and decreases drip loss of broiler breast meat. *Poultry Science*, 87(3): 480-484.
- Choi, Y. S., Hwang, K. E., Jeong, T. J., Kim, Y. B., Jeon, K. H., Kim, E. M., & Kim, C. J. (2016). Comparative study on the effects of boiling, steaming, grilling, microwaving and superheated steaming on quality characteristics of marinated chicken steak. *Korean Journal for Food Science of Animal Resources*, 36 (1): 1-7.
- Department of Agriculture, Forestry & Fisheries (DAFF). (2006). Meat Safety Act (Act no. 20 of 2000, R153/2006). South African Government Gazette. 28520:20-22.
- <https://www.nda.agric.za/vetweb/Legislation/Meat%20safety/Poultry%20Regulations.pdf>

- El-Bahr, S., Shousha, S., Shehab, A., Khattab, W., Ahmed-Farid, O., Sabike, I., ... & Albosadah, K. (2020). Effect of dietary microalgae on growth performance, profiles of amino and fatty acids, antioxidant status, and meat quality of broiler chickens. *Animals*, 10(5): 761.
- Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49(4): 447-457.
- Ježek, František, Josef Kameník, Blanka Macharáčková, Kateřina Bogdanovičová, and Jiří Bednář, (2020). "Cooking of meat: effect on texture, cooking loss and microbiological quality—a review." *Acta Veterinaria Brno*, 88 (4): 487-496.
- Juárez, M., Aldai, N., López-Campos, Ó., Dugan, M. E. R., Uttaro, B., & Aalhus, J. L. (2012). Beef texture and juiciness. *Handbook of meat and meat processing*, 9: 177-206.
- Kandeepan, G. (2009). Feeding regiments affecting meat quality characteristics. *MESO: Prvi hrvatski časopis o mesu*, 11(4), 240-249.
- Kralik, G., Kralik, Z., Grčević, M., & Hanžek, D. (2018). Quality of chicken meat. *Animal Husbandry and Nutrition*. Chapter 4:64-93.
- Liu, J., Ellies-Oury, M. P., Stoyanchev, T., & Hocquette, J. F. (2022). Consumer perception of beef quality and how to control, improve and predict it? Focus on eating quality. *Foods*, 11(12): 1732.
- Marangoni, F., Corsello, G., Cricelli, C., Ferrara, N., Ghiselli, A., Lucchin, L., & Poli,

- A. (2015). Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: An Italian consensus document. *Food and Nutrition Research*, 59: 1–11.
- Mariey, Y. A., Samak, H. R., & Ibrahim, M. A. (2012). Effect of using *Spirulina platensis* algae as a feed additive for poultry diets: 1-productive and reproductive performances of local laying hens. *Egyptian Poultry Science Journal*, 32(1): 201–215.
- Masaire, E., Madzingira, O., Samkange, A., Kandiwa, E., Mushonga, B., & As, B. (2018). Characterization of poultry production and management systems in the communal areas of Namibia. *African Journal of Poultry Farming*, 6(3): 265–276.
- Melesse, A. (2014). Significance of scavenging chicken production in the rural community of Africa for enhanced food security. *World's Poultry Science Journal*, 70: 593–606.
- Moustafa, E.S.; Alsanie, W.F.; Gaber, A.; Kamel, N.N.; Alaqil, A.A.; Abbas, A.O. (2021). Blue-Green Algae (*Spirulina platensis*) Alleviates the Negative Impact of Heat Stress on Broiler Production Performance and Redox Status. *Animals*, 11 (1243):1-13.
- Savell, J. W., & Cross, H. R. (1988). The role of fat in the palatability of beef, pork, and lamb. *Designing foods: Animal product options in the marketplace*, 345.
- Sebola, N. A., Mlambo, V., Mokoboki, H.K., & Muchenje, V. (2015). Growth performance and carcass characteristics of three chicken strains in response to

incremental levels of dietary *Moringa oleifera* leaf meal. *Livestock Science*, 178:202-208.

Packard, R. (2014). *Comparison of production parameters and meat quality characteristics of South African indigenous chickens* (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Park, J. H., Lee, S. I., & Kim, I. H. (2018). Effect of dietary *Spirulina* (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission and breast meat quality of broiler chickens. *Journal of Poultry Science*, 97:2451-2459.

Pestana, J.M., Puerta, B., Santos, H., Madeira, M. S., Lopes, P. A., Pinto, R. M. A., Lemos, J. P. C., Fontes, C. M. C. A., Lordelo, M. M., & Prates, J. A. M. (2020). Impact of dietary incorporation of *Spirulina* (*Arthrospira platensis*) and exogenous enzyme on broiler performance, carcass traits and meat quality. *Poultry Science*, 99 (5): 1-14.

Petrus, N. P. (2011). *Characterisation and production performance of indigenous chickens in northern Namibia regions*. PhD dissertation. University of Namibia, Namibia.

Pieterse, E., Erasmus, S. W., Uushona, T., & Hoffman, L. C. (2019). Black soldier fly (*Hermetia illucens*) pre-pupae meal as a dietary protein source for broiler production ensures a tasty chicken with standard meat quality for every pot. *Journal of the Science of Food and Agriculture*, 99(2): 893-903.

- Raach-Moujahed, A., Hassani, S., Zairi, S., Bouallegue, M., Darej, C., Haddad, B., & Damergi, C. (2011). Effect of dehydrated *Spirulina platensis* on performances and meat quality of broilers. *Research Opinions in Animal and Veterinary Sciences, 1*(8): 505-509.
- Rajmohan, D. K. D. (2017). *Use of Ionic Gelation to Reduce Perceived Bitterness of Spirulina Protein* (Doctoral dissertation, Oklahoma State University).
- Rubel, M. (2018). Effects of dietary supplementation of algae (*Spirulina plantensis*) as an alternative to antibiotic on growth performance and health status of broiler chicken. M.Sc thesis. Sher-el-Bangla Agricultural University. Dhaka.
- Taelman, S. E., De Meester, S., Van Dijk, W., Da Silva, V., & Dewulf, J. (2015). Environmental sustainability analysis of a protein-rich livestock feed ingredient in The Netherlands: Microalgae production versus soybean import. *Resources, Conservation and Recycling, 101*: 61-72.
- Uushona, T. (2015). Black soldier fly (*Hermetia illucens*) pre-pupae as a protein source for broiler production. M.Sc. Thesis . University of Stellenbosch. South Africa.
- Van Marle-Koster, E., & Webb, E. C. (2000). Carcass characteristics of South African native chicken lines. *South African Journal of Animal Science, 30*(1): 53-56.
- Vonshak, A. (2002). Use of *Spirulina* Biomass, In: Vonshak A. (ed.), *Spirulina platensis (Arthrospira) Physiology Cell Biology and Biotechnology*, Taylor & Francis, ISBN 0-203-48396-0, London. pp. 159-173.
- Watanabe, G., Motoyama, M., Nakajima, I., & Sasaki, K. (2018). Relationship between

water-holding capacity and intramuscular fat content in Japanese commercial pork loin. *Asian-Australasian Journal of Animal Sciences*, 31(6): 914.

Zhuang, H., Bowker, B. C., & Savage, E. M. (2017). Assessment of Juiciness Intensity of Cooked Chicken Pectoralis Major. *Food Nutrition Journal*, 2: 152.

## CHAPTER 7

### GENERAL CONCLUSION AND RECOMMENDATIONS

#### 7.1 Conclusions

Spirulina has become a trend in the food and feed industry as it is considered to be rich in essential nutrients especially proteins for humans and animals. Therefore, the general objective of this study was to determine the effect of feeding incremental levels of *Spirulina platensis* as a protein source on feed intake, growth performance, carcass yield, intestinal gut microflora, meat quality and sensory attributes of Boschveld and Potchefstroom Koekoek chickens. Firstly, the inclusion of Spirulina in the current study reduced feed intake of the chickens. The low feed intake in chickens fed Spirulina diets was associated with the presence of phenolic compounds such as tannins that causes strong smell of Spirulina and its tendency to stick in the mouth of the chickens. This was because flavor of feed affects feed intake as birds are more responsive to weakly flavored feeds than strongly flavored feed, as their taste buds gets overwhelmed (Gentle, 1985). However, feed intake increased with time within the treatments as expected with increasing age of birds (Sebola *et al.*, 2015). Chickens fed control diet recorded heavy weights. This may be attributed to high feed intake of chickens on control diet than those on Spirulina diet. The amount of feed consumed is related to the performance of meat-type poultry as feed intake influences body weight gain (Ferket & Gernat, 2006). The Potchefstroom Koekoek chickens attained their peak intake much earlier than Boschveld chickens; hence more nutrients were available for growth in Boschveld chickens which may have contributed to higher average daily gain in Boschveld than in

Potchefstroom Koekoek. Increase in Spirulina in the diets increased feed conversion ratio, which means, more feed needed to be consumed for the chickens to convert feed into body weight. This indicated better utilization of the Spirulina diet by the Boschveld at an older age. The higher crude protein concentration in Spirulina affected the crude protein intake of the chickens. Increase in Spirulina level in the diet resulted in decrease in the age at which peak crude protein intake was reached. The chickens fed Spirulina diet had low protein efficiency ratio compared to control group, which could be attributed to higher energy expenditure to excrete the products of protein metabolism (Gous *et al.*, 2018).

Secondly, there were differences in the breast muscle and thigh weight due to treatment diets. Chickens on control diet recorded heavy breast muscle and thigh weight than those on Spirulina diets. The differences in the breast muscle and thigh weight were linked to the composition of the diets and the slaughter weight of the chickens which is directly linked to feed intake (Mullenix *et al.*, 2022). Potchefstroom Koekoek had a higher spleen size compare to Boschveld which is evidence that spleen size is related to body weight because Potchefstroom Koekoek is a heavy breed.

Thirdly, a healthy normal flora is the first line of defense for all animal bodies to invading pathogens (Hofacre *et al.*, 2001). While that is true, it is reported that the health and performance of poultry is influenced by gastrointestinal normal flora (Yusuf *et al.*, 2016). Thus, the third objective of this study evaluated the effect of incremental levels of Spirulina on intestinal gut microflora. The coliform counts decreased with the increase in Spirulina in the diets, while *Lactobacillus* counts increased with increase in Spirulina in the diets. The increase in *Lactobacillus* count can also be explained by the

probiotics properties of Spirulina which promotes the inhibition of pathogenic bacteria growth such as coliforms while promoting the growth of probiotic colonizing species such as *Lactobacillus sp.* (Ansari *et al.*, 2018). The decrease in coliforms with an increase in Spirulina can also be associated with the antibacterial activities of Spirulina against coliforms due to the presence of active ingredients such as alkaloids and lipopolysaccharides (Park *et al.*, 2018).

Lastly, the study looked at the effect of feeding incremental levels of Spirulina on meat quality and sensory attributes because the viability of a potential chicken feed depends on its effect on the quality of the end product which is meat or eggs. There were no significant differences found in the meat quality parameters assessed. However, the panelists discriminated some eating quality attributes with dietary treatments by which diet significantly influenced the flavor, initial juiciness and sustained juiciness of the chicken meat. Meat from control group (0%) had a prominent chicken flavor than meat from Spirulina fed groups (5%, 10% & 15%). Initial juiciness and sustained juiciness was more in meat from control group than meat from Spirulina groups.

## 7.2. Recommendations

From the results of this study, it was observed that the inclusion of Spirulina at 5% in the diet resulted almost in similar results with the diet that only had soybean as a protein source in most of the assessed parameters. Based on these results, it is therefore best if only up to 5% Spirulina can be included in the diets for indigenous (dual purpose) chickens. However, due to the strong odour of *Spirulina platensis* which affects feed intake, meat flavor and acceptability of the meat; this must be further investigated to find ways to deodorize Spirulina before including it at high levels in the chicken feeds at a large scale. The study further recommends that for further studies, the gender of the chickens be considered and be evaluated differently at each inclusion level for carcass cuts yield, internal organ sizes and meat quality. In order to improve the intestinal health for chickens, this study recommends the inclusion of Spirulina in the chicken diets due to probiotics properties of Spirulina which promotes the inhibition of pathogenic bacteria growth while promoting the growth of probiotic colonizing species. However, further researches are recommended to establish the exact amount of Spirulina that can be safely incorporated in the chicken`s diets (starter, grower and finisher) that will improve both the feed intake, growth performance and gut microflora without compromising on one of the parameters. Most importantly, further researches are recommended to find out the cost benefit analysis of using Spirulina as a protein source than soybean, which then determines whether it is better to use Spirulina rather than soybean.

### 7.3. References

- Ansari, M. S, Hajati, H., Gholizadeh, F., Soltani, N.,& Alavi, M. (2018). Effect of different levels of spirulina platensis on growth performance, intestinal morphology, gut microflora, carcass characteristics and some blood parameters in broiler chickens. *Journal of Phycological Research*, 2(2): 187-197.
- Gentle, M.J. (1985). Sensory involvement in the control of food intake in poultry. *Proceedings of the Nutrition Society*, 44: 313-221.
- Gous, R. M., Faulkner, A. S., & Swatson, H. K. (2018). The effect of dietary energy: protein ratio, protein quality and food allocation on the efficiency of utilisation of protein by broiler chickens. *British poultry science*, 59(1): 100-109.
- Hofacre, C. L., White, D. G., Maurer, J. J., Morales, C., Lobsinger, C., & Hudson, C. (2001). Characterization of antibiotic-resistant bacteria in rendered animal products. *Avian Diseases*, 953-961.
- Ferket, P.R., and Gernat, A.G. (2006). Factors that affect feed intake of meat birds: A review. *Intenational Journal of Poultry Science*, 5(10) :905-911.
- Mullenix, G. T., Maynard, C. J., Owens, C. M., Rochell, S. J., Bottje, W. G., Brister, R. D., & Kidd, M. T. (2022). Spirulina platensis meal inclusion effects on broilers fed a reduced protein diet. *Journal of Applied Poultry Research*, 31: 100199.
- Park, J. H., Lee, S. I., & Kim, I. H. (2018). Effect of dietary Spirulina (Arthrospira) platensis on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission and breast meat quality

of broiler chickens. *Journal of Poultry Science*, 97:2451-2459.

Sebola, N. A., Mlambo, V., Mokoboki, H.K., & Muchenje, V. (2015). Growth performance and carcass characteristics of three chicken strains in response to incremental levels of dietary *Moringa oleifera* leaf meal. *Journal of Livestock Science*, 178:202-208.

Yusuf, M. S., Hassan, M. A., Abdel-Daim, M. M., El Nabtiti, A. S., Ahmed, A. M., Moawed, S. A., & Cui, H. (2016). Value added by *Spirulina platensis* in two different diets on growth performance, gut microbiota, and meat quality of Japanese quails. *Veterinary World*, 9(11): 1287.

## APPENDICES

### Appendix A: Ethical clearance certificate



#### ETHICAL CLEARANCE CERTIFICATE

**Ethical Clearance Reference Number: FANR/004/2019 Date: 13 November 2019**

This Ethical Clearance Certificate is issued by the University of Namibia Animal Research Ethics Committee (AREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Research & Publications Committee sitting with the Postgraduate Studies Committee.

**Title of Project:** Assessing the potential of *Spirulina Platensis* as a protein source and its effect on feed intake, growth performance, carcass yield and meat quality of two indigenous chicken breeds.

**Nature/Level of Project:** Master of Science in Agriculture (Animal Science)

**Researcher:** Aino Nambahu

**Student Number:** 200971981

**Faculty:** Faculty of Agriculture and Natural Resources

#### **Supervisors:**

Take note of the following:

- (a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the AREC. An application to make amendments may be necessary.
- (b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the AREC.
- (c) The Principal Researcher must report issues of ethical compliance to the AREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by AREC.
- (d) The AREC retains the right to:
  - (i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
  - (ii) Request for an ethical compliance report at any point during the course of the research.

REC wishes you the best in your research.

REC Chairperson

Manfred Janik

A handwritten signature in black ink, appearing to be "M. Janik", written over a horizontal line.

## Appendix B: Sensory attributes

University of Namibia: Faculty of Agriculture, Engineering and Natural Sciences  
Department of Animal production, Agribusiness and Economics

### Attributes for descriptive sensory evaluation

#### 1. Aroma

##### Aromatic attributes

##### Description

Overall aroma intensity

Intensity of aroma in first few sniffs

#### 2. Flavour

##### Flavour attributes

##### Description

Chicken flavor

Flavour associated with the meat from Chicken

Sour taste

Taste associated with a citric acid solution

Bitter taste

Taste associated with caffeine or dark chocolate.

#### 3. Texture

##### Texture (taste) attributes

##### Description

Initial juiciness

Amount of fluid extruded on surface of meat sample when pressed between the thumb and forefinger (pressed perpendicular to fibres)

Sustained juiciness

Amount of moisture perceived during mastication

Tenderness

Impression of tenderness after mastication

Mealiness

Disintegration of muscle fibre where mealiness disintegrates into very small particles

## **Appendix C: Sensory evaluation questionnaire**

**University of Namibia**

**Faculty of Agriculture, Engineering and Natural Sciences**

**Department of Animal production, Agribusiness and Economics**

Score sheet for descriptive sensory evaluation training: Influence of feed composition on chicken meat texture, flavor and aroma.

Panel leader: Aino Nambahu

Date:

Form number: 1

### Instructions

1. Rinse your mouth with water and with any other item provided for rinsing, before and after each sample.
2. Rest for at least 30 seconds before proceeding to the next sample.
3. Answer by indicating your score in the rating column provided.

### **1. AROMA**

**1. How would you rate the overall aroma intensity of the meat?**

Sample A  
code

Rating

0 1 2 3 4 5 6 7 8 9 10

None

Prominent



**2. FLAVOURS**

**1. How would you rate the chicken flavor of the meat?**

Sample A  
code

Rating

0 1 2 3 4 5 6 7 8 9 10

None

Prominent



**2. How would you rate the sour taste of the meat?**

Sample A  
code

Rating

0 1 2 3 4 5 6 7 8 9 10

None

Prominent



**3. How would you rate the bitter taste of the meat?**



**4. TEXTURE**

**1. How would you rate the initial juiciness of the meat?**

Sample code A



**2. How would you rate the sustained juiciness of the meat?**

Sample A  
code



**3. How would you rate the tenderness of the meat?**

