Effect of different dietary fibre raw material sources on production and gut development in fast-growing broilers

by

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DECLARATION

I, Andries Benjamin Fourie, hereby declare that this dissertation, which I hereby submit for the MMedVet (Altil) degree to the Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, is my own work and that it has not been previously presented by me for degree purposes at any other tertiary institution.

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A.B. FOURIE

DEDICATION

To my wife, Dorette, and son, HP. I forfeited much family time to invest in this, which wouldn't have been possible without your continued support and understanding.

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ABSTRACT

EFFECT OF DIFFERENT DIETARY FIBRE RAW MATERIAL SOURCES ON PRODUCTION AND GUT DEVELOPMENT IN FAST-GROWING BROILERS

by

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Supervisor: Dr. D.B.R. Wandrag

Many recent studies have been published about the beneficial effect of different fibre sources in broiler diets. To assess these effects with raw material sources available in Southern Africa, a trial was done with four treatments; control diet, 2% sunflower hulls, 2% malt culms and 0.8% of a commercial lignocellulose product (OptiCell®).

Using a completely randomised block design, each treatment had 24 repetitions (96 pens in total), with 48 birds per pen. The effects were measured weekly (days 7, 14, 21, 28 and 32) by assessing production parameters, gut development and the humoral immune response. Production parameters were measured per pen, gut measurements were done on 12 birds per treatment each week, and humoral immune response on 24 birds per treatment at 32 days.

The promising responses seen on other fibre sources such as sugar beet pulp and oat hulls, were not achieved here with the local fibre sources. There were no significant differences from the control group based on production parameters. Concerning gut development, sunflower hulls produced a significant improvement compared to the control group with regards to caecal and overall intestinal lengths at 7 days. There was a numerical improvement in gizzard weights at 7 days for the malt culms group. No significant differences were detected on the serology.

This shows that there may be merit in including sunflower hulls in the pre-starter period (days 0-7), although it did not translate to production advantages here. Different inclusion levels could be trialed.

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ABBREVIATIONS

ADG	Average Daily Gain
AH	Acid Hydrolysis
CAL	Central Analytical Laboratories
cm	Centimetres
EE	Ether Extract
ELISA	Enzyme-Linked Immunosorbent Assay
FCR	Feed Conversion Ratio
g	Grams
GALT	Gut-Associated Lymphoid Tissue
GFC	Grain Field Chickens
HVT	Herpes Virus of Turkey
IBD	Infectious Bursal Disease
MDCP	Monodicalcium Phosphate
NDV	Newcastle Disease Virus
NSP	Non-starch polysaccharides
ppm	Parts Per Million
TX	Texas
USA	United States of America

CHAPTER 1: BACKGROUND

1.1 Introduction

Due to the raw materials used in broiler chicken diets, fibres are always a part of these diets, but in contrast to ruminants their role in broilers is not always that clear. Fibre is not at the forefront in an environment where we are attempting to get maximum production from these birds in as short a time and with as little feed as possible; that typically falls more within the domains of protein (amino acids) and energy.

Dietary fibre has been recognized as an essential ingredient having beneficial effects on gut health and a balanced gut flora (Neufeld, 2010). Dietary fibre is a form of carbohydrate made up of the largely indigestible parts of plants, passing through the small intestine relatively unchanged (Stribling and Ibrahim, 2023). Chemically it is considered as the sum of non-starch polysaccharides (NSP) and lignin (Theander et al., 1994). The NSP can be divided into two sub-groups, namely water-soluble and insoluble fibre (Raninen et al., 2011). The water-soluble fibre increases gut viscosity resulting in a slower feed passage rate and are mostly fermented in the large intestine and caecae. The insoluble fibre increases the rate of feed passage and are non-fermentable (Sozcu, 2019, Rohe et al., 2020). Lignocellulose largely consists of insoluble cellulose, hemicellulose and phenolic lignin (Rohe et al., 2020).

The non-fermentable fibre which is inert to digestive enzymes in the upper gastrointestinal tract (e.g., cellulose and lignin), plays an important role in intestinal transit. This fibre induces contractions which allows peristalsis, and has a laxative effect by mechanical stimulation of the intestines, promoting mucous secretion (Stribling and Ibrahim, 2023). It also tends to stay in the upper gastrointestinal tract for longer, mechanically stimulating gizzard muscles to develop (Jiménez-Moreno et al., 2019).

Fermentable fibre is fermented in the large intestine by bacteria, stabilizing the natural gut flora and assisting in the control of certain pathogens (Neufeld, 2010). Some of these

fermentation by-products such as butyric acid can be an important energy source for colonocytes. Butyrate also has anti-inflammatory effects and can strengthen the mucosal barrier integrity by upregulating the expression of tight junction proteins (Van Immerseel et al., 2010).

South Africa slaughters about 21 million broiler chickens per week (SAPA, 2022). To feed this large number of broilers, a massive feed industry is needed. With feed costs contributing about 65% to the cost of broiler production, this feed needs to be optimally balanced to maximise production efficiency. It is a constant drive for perfection, with persistent evolution of diets as new research becomes industry norms. Financial viability is paramount for animal production, therefore production parameters were the primary focus for this trial. Secondly, due to gastrointestinal development potentially being a contributing factor to better production, this was also measured. Lastly due to trials showing promise on the immunomodulatory front (Saadatmand et al., 2019, Sabour et al., 2019), parameters were also measured to assess this.

Southern African broiler producers typically use breeds classified as fast-growing broilers. In general broiler breeds with mean growth rates of more than 50 grams per day are classified as fast-growing broilers, with those growing less than 50 grams per day regarded as slow-growing broilers. While fast-growing broilers dominate the Southern African region, the slow-growing breeds have a foothold in the European market due to perceived improved animal welfare for birds growing slower (Rayner et al., 2020).

1.2 Literature review

Every year an increasing number of studies are published where the effect of different fibre sources is measured in broilers. The different fibre sources on their own generally have very low nutritive values. Where their benefits come to the fore is when focusing specifically on the gastrointestinal tract, ranging from the actual morphology of cells to possible immunomodulatory effects. By means of altering the physiological processes in

broilers' guts such as gizzard development, digestive enzyme activities and an improved antioxidant status, insoluble fibre may improve the utilization of nutrients (Shang et al., 2020).

Most of these studies have been done on fibre sources not readily available in sufficient quantities in Southern Africa, such as oat hulls and sugar beet pulp. For this study the focus was thus on fibre more readily available locally, being sunflower hulls, malt culms, and a commercial lignocellulose product.

Sunflower hulls show positive results in scientific studies and are easy to source in Southern Africa. Moradi et al. (2020) specifically looked at the effects until 21 days of age, and showed that inclusion of sunflower hulls improved the birds' body weights and feed conversion ratio (FCR), outperforming the rice hull and lignocellulose rations. Furthermore, it reduced the gizzard's pH in combination with coarser corn, and produced higher gizzard weights. This reduction in gizzard pH is attributable to fibre particles being retained for longer in the gizzard, with hydrochloric acid production being stimulated by mechanoreceptors (Jimenez-Moreno et al., 2009c, Duke, 1986). An added benefit is that most *Salmonella* species are inhibited at reduced pH's (Cox et al., 1972).

In another trial (Jamshidi and Moradi, 2020) sunflower hulls were included at 3%. This resulted in improved body weights up to 21 days of age. It increased gizzard and jejunum weights when presented in a coarse form. In contrast to the previous study, the gizzard pH was unaffected. Rice hulls and camelina hulls both led to higher jejunal weights. It was noted that with all 3 fibre inclusions the coarser particles (3mm) yielded higher caecal weights compared to finer particles (1mm).

Where a 2.5% and 5% inclusion rate was fed, inconsistent effects were seen on the gastrointestinal tract measurements (Jiménez-Moreno et al., 2019). It decreased gizzard pH and increased gizzard weights. It also improved nutrient digestibility by increasing the secretion of endogenous enzymes, with the 2.5% fibre inclusion being most favourable. The other fibre sources considered were oat hulls and rice hulls, with oat hulls additionally improving nutrient retention.

Tejeda and Kim (2020) trialled purified cellulose and soybean hulls, included to reach crude fibre values of 4%, 6% and 8% in the diets. All soybean hull diets showed heavier gizzard and intestine weights. At 20 days of age duodenal villi height improvements were seen (8% inclusion), with ileal villi heights also higher in both 4% crude fibre diets. Finally, the soybean hull diets also showed improved amino acid digestibility.

Wheat bran was used in broilers grown to 42 days (Shang et al., 2020), with multiple parameters being improved. This ranged from increased digestibility to lower serum cholesterol, increased amylase and trypsin activity, increased gizzard weights, increased villus height in the jejunum and ileum, and an improved antioxidant status.

Where lignocellulose (an insoluble constituent of plant cell walls) was used in slow-growing broilers, it caused impaired ileal digestibility of protein when 10% lignocellulose was included in the diet. Despite this, the birds' performance was unaffected at all three inclusion levels trialled (0.8%, 5% and 10% lignocellulose). This might be explained by the fact that diets were not diluted with the additional lignocellulose, but formulated to be isocaloric (Rohe et al., 2020). An advantage of lignocellulose products is that they show a lower risk of being contaminated with mycotoxins or bacteria than fibre sources originating from crops. Due to their high crude fibre values they can also be added at low inclusion rates, allowing for flexibility in formulation of the diets (Neufeld, 2010).

In a trial with sugar beet pulp and rice hulls included at 3% (Sadeghi et al., 2015), both fibre sources improved antibody titres against Newcastle Disease Virus (NDV) vaccine. This humoral immune response was also seen by Sabour et al. (2019), using sugar beet pulp and rice hulls at 3% inclusion levels, in combination with organic acids in broilers grown to 42 days. Although performance improvements were not seen, the level of antibodies produced against Avian Influenza vaccine increased with the rice hull and organic acid combination.

Saadatmand et al. (2019) showed a similar response in broilers, where sugar beet pulp in combination with threonine at 110% of recommended levels, also provided an

improvement in humoral immunity. In this study the rice hulls and sugar beet pulp were included at 3% in their respective treatment groups; both treatment groups decreased the birds' feed intake, coupled with increased weight gains.

A possible explanation for this positive immune response is that the fibre aids in creating a stable commensal microflora, which in turn could have an interactive effect on the gut associated lymphoid tissue (GALT) (Montagne et al., 2003), with a beneficial effect on the bird's entire immune system. Serum antibody levels are an indication of the bird's resistance to a disease. These findings suggest that additional fibre in broiler diets indirectly improves protection against diseases which are vaccinated against, by increased antibody production.

Table 1 summarises different fibre sources tested in broilers, with the effect that these fibre sources had on different parameters. In addition to the sources mentioned above, data was also used from other published trials (Gonzalez-Alvarado et al., 2007, González-Alvarado et al., 2008, Jimenez-Moreno et al., 2016, Jimenez-Moreno et al., 2009a, Jimenez-Moreno et al., 2010, Jimenez-Moreno et al., 2009b).

This illustrates sunflower hulls to be a good option to trial, determining whether the results can be replicated locally with this readily available product. Lignocellulose was chosen for its lower health risk and the uniformity of the commercial product, even though there is not an abundance of published data. Malt culms were opted for due to the availability, although its relatively lower crude fibre content, compared to other fibre sources, probably excluded it from other trials.

Considering the data available as well as local experiences, an inclusion level of 2% (weight) was used for sunflower hulls and malt culms. OptiCell® was included at the manufacturer's recommended level of 0.8%.

Table 1: Summary of the effect of different fibre sources on different parameters.

Product	**Gizzard weight**	**Gizzard pH**	**Body weight**	**FCR**	**Humoral immunity**
Oat hulls	+[d]+[i]+[j]+[l]+[m]+[n]	+[d]+[l]+[m]+[n]+[o]	+[i]+[k]+[m]+[n]	+[i]+[k]+[m]+[n]	
Sunflower hulls	+[b]+[c]+[d]	+[b]+[d]	+[b]+[c]+[k]	+[b]+[k]	
Sugar beet pulp	+[m]+[n]	+[l]+[m]+[n]	+[m]+[n] -[f]-[h]	+[m]+[n] -[f]-[h]	+[f]+[h]
Soybean hulls	+[e]+[i]	+[o]	+[i]	+[i]	
Rice hulls	+[d]	+[d]	+[k] -[f]-[h]	+[h]+[k] -[h]	+[f]+[g]
Lignocellulose	+[b]		+[b]		
Wheat bran	+[a]				
Malt culms					

A "**+**" sign represents a study found to positively influence the parameter, while a "**-**" sign represents a negative influence. Empty blocks are indicative of a lack of available data to assess the parameter.

References are indicated by the following superscripts in the table: [a] (Shang et al., 2020); [b] (Moradi et al., 2020); [c] (Jamshidi and Moradi, 2020); [d] (Jiménez-Moreno et al., 2019); [e] (Tejeda and Kim, 2020); [f] (Sadeghi et al., 2015); [g] (Sabour et al., 2019); [h] (Saadatmand et al., 2019); [i] (Gonzalez-Alvarado et al., 2007); [j] (González-Alvarado et al., 2008); [k] (Jimenez-Moreno et al., 2016); [l] (Jimenez-Moreno et al., 2009a); [m] (Jimenez-Moreno et al., 2010); [n] (Jimenez-Moreno et al., 2009b); [o] (Jimenez-Moreno et al., 2009c).

CHAPTER 2: HYPOTHESIS

2.1 Problem

As shown in Table 1, there are many studies which indicate that fibre sources can improve production and gut development in fast-growing broilers, despite their low nutritive values. These fibre sources improve development of especially the upper gastrointestinal tract and can also have an immunomodulatory effect by altering the gut microflora and its interaction with the GALT. Most of these studies have been done on fibre raw materials which are not readily available in Southern Africa e.g., oat hulls and sugar beet pulp. The aim was to focus this study using locally available fibre sources such as sunflower hulls, malt culms and OptiCell® (Agromed distributed by Vitam International).

In addition to the parameters in Table 1, some other parameters were also included in this trial. Mortality rate was included due to its ease in measurement together with the other production parameters. Gizzard erosion scores were added to assess whether altered gizzard pH would influence the gizzard's integrity. Intestinal length was measured to macroscopically assess whether there are differences in development of the intestinal tract. Finally, bursal weights were taken to ensure that differing serological titres weren't due to differences in bursal health.

2.2 Hypothesis

Inclusion of sunflower hulls, malt culms or OptiCell® as dietary fibre raw material sources for fast-growing broilers, will result in the improvement of production parameters, intestinal health and the immune system compared to a control diet with no additional fibre sources.

2.3 Objectives

The objectives of this research were to measure the effect of no additional fibre (control), the inclusion of 2% sunflower hulls, 2% malt culms and 0.8% OptiCell® on:

- Production parameters: live body weight, average daily gain (ADG), feed conversion ratio (FCR) and mortality.
- Intestinal health: gizzard pH, gizzard erosions, gizzard weights, total intestinal and caecal lengths.
- The immune system: Bursa of Fabricius weights and Enzyme-Linked Immunosorbent Assay (ELISA) titres in response to Newcastle Disease Virus (NDV) vaccination.

CHAPTER 3: EXPERIMENTAL DESIGN

3.1 Materials

3.1.1 Grain Field Chickens Workers' Trust Trial Pens

The trial facility named GFC Workers' Trust, is located at the Dorpsgronde of Reitz in the Free State Province, being the property of Grain Field Chickens.

The trial pens were arranged in a line along the middle of a commercial broiler house, extending for about 70% of the length of the house. On either side of this line extending along the whole house, were two separate camps also stocked with broilers of the same age, to mimic commercial full house conditions in the industry. This is illustrated in Figure 1. Litter for this trial consisted of pine shavings.

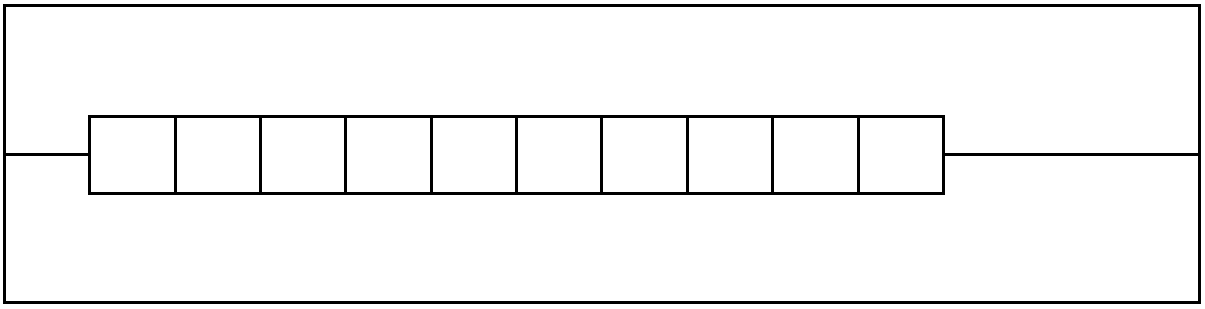

Figure 1: Illustration of the house from the top, with trial pens and partition fence along the middle.

The trial house consists of 96 pens, each able to house 48 birds. This gives a total of 4 608 birds.

3.1.2 Chicks

Day-old chicks (n = 4 608) were sourced from Eagle's Pride Hatchery. All chicks were sexed with only males used in the trial to limit variability. The parents were a 46-week old Ross 308 flock.

All birds received the same vaccination program:

- Hatchery administration
    - Live NDV vaccine, sprayed
    - HVT vectored Newcastle Disease vaccine, subcutaneous injection
    - Live Infectious Bursal Disease (IBD) vaccine, subcutaneous injection
    - Live Mass-type and 793B-type Infectious Bronchitis vaccines, sprayed
- Day 10 on-farm administration
    - Live 793B-type Infectious Bronchitis vaccine, sprayed

3.1.3 Feed

All feed was manufactured by Simple Grow in Centurion, South Africa.

3.1.3.1 Dietary fibre raw material sources

3.1.3.1.1 Control diet

No additional fibre sources were included.

3.1.3.1.2 Sunflower hulls

The sunflower hulls were ungrounded at a 2% inclusion level, with a crude fibre value of 49.8%.

3.1.3.1.3 Malt culms

The malt culms were also ungrounded at a 2% inclusion level, with a crude fibre value of 15%. This is a by-product from the beer fermentation process.

3.1.3.1.4 OptiCell®

OptiCell®, a commercial lignocellulose product, was used for the trial. It has the benefits of being homogenous, of a consistent quality and available supply throughout all seasons.

This is a modern lignocellulose product which has a standard combination of fermentable and non-fermentable fibres. The non-fermentable fibre has a physical action in the gastrointestinal tract, while the fermentable fibre serves as a food source for bacteria producing short chain fatty acids, especially butyrate.

This product was included at the recommended 0.8%, with a crude fibre value of about 59%. It is a natural lignocellulose product made from fresh wood, with the crumbles having a size of 0.4 – 1.6 mm.

3.1.3.2 Rations

All four rations were formulated to be isocaloric, with each fibre product included on their full matrix values – not added on top. These inclusions applied to all five phases within a ration: pre-starter (0-7 days), starter (7-14 days), grower (14-21 days), finisher (21-28 days) and post-finisher (28-32 days).

The formulated dietary values for all diets are indicated in Table 2. The crude fibre values were the only major differences, indicated for each diet in Table 3.

Table 2: Formulated percentage values for each major dietary parameter.

	Pre-starter	**Starter**	**Grower**	**Finisher**	**Post-Finisher**
Moisture	10,94	11,15	11,08	10,94	10,89
Protein	22,86	21,25	20,23	19,23	19,30
Fat (EE)	5,09	5,17	5,65	6,68	7,20
Fat (AH)	5,77	5,73	6,23	7,26	7,78
Calcium	0,94	0,87	0,76	0,68	0,68
Phosphorus	0,64	0,51	0,43	0,35	0,35
Sodium	0,17	0,15	0,14	0,14	0,14
Chloride	0,30	0,30	0,25	0,25	0,25
Potassium	0,86	0,87	0,83	0,78	0,79

EE – Ether Extract; AH – Acid Hydrolysis.

Table 3: Percentage crude fibre values for each ration.

	Pre-starter	**Starter**	**Grower**	**Finisher**	**Post-Finisher**
Control	3,85	3,91	3,75	3,57	3,58
Sunflower hulls	4,82	4,88	4,72	4,54	4,54
Malt culms	4,05	4,11	3,94	3,77	3,77
OptiCell®	4,31	4,37	4,21	4,03	4,04

All 4 rations had Flavophospholipol (Animate) included at 12 ppm in all 5 phases for clostridial control, as well as Lasalocid (Zoetis) at 75 ppm for coccidiosis control.

The control ration's formulation is indicated in Table 4 for each phase of the ration.

Table 4: Control ration percentage inclusions of each ingredient.

	Pre-starter	Starter	Grower	Finisher	Post-Finisher
Maize	49,52	52,77	55,79	57,97	57,10
Soya oilcake	30,77	29,6	25	19,77	20,13
Full fat soya	8	10	12	15	15
Sunflower oilcake	2	2	2	2	2
Gluten 60	3	0	0	0	0
Oil crude soya coater	1,37	0,98	1,04	1,48	2,03
Feed lime	1,22	1,31	1,15	1,09	1,09
MDCP	1,49	0,83	0,50	0,16	0,16
Salt (fine)	0,30	0,32	0,23	0,23	0,24
Sodium bicarbonate	0,17	0,06	0,15	0,16	0,15
Methionine (84%)	0,31	0,34	0,36	0,34	0,34
Lysine (78%)	0,28	0,20	0,21	0,22	0,20
Choline (60%)	0,20	0,20	0,20	0,20	0,20
Threonine (98%)	0,15	0,15	0,13	0,13	0,10
Valine	0	0,01	0,025	0,028	0,028
Lasalocid (15%)	0,05	0,05	0,05	0,05	0,05
Flavomycin (8%)	0,015	0,015	0,015	0,015	0,015
Pellibond	1,00	1,00	1,00	1,00	1,00
Premix (including phytase and mannanase)	0,16	0,16	0,16	0,16	0,16

MDCP – Monodicalcium Phosphate.

3.2 Methods

3.2.1 Introduction

The trial was divided into four groups with each of the 96 pens representing one treatment. Each group thus had 24 repetitions, totaling 1 152 birds per group.

Each pen was stocked with 51 chicks at placement, reduced to 48 chicks at 7 days. This was done to improve uniformity by removing the non-starter or weakest birds. The final

stocking density was thus 24 birds per m^2, mimicking the highest stocking densities experienced under commercial conditions in Southern Africa.

3.2.2 Assignment of treatments to different pens

A completely randomised block design was used, generated in Microsoft Excel and illustrated in Table 5.

Table 5: Record of treatments allocated to specific numbered pens.

PEN	BLOCK	TREATMENT
1	1	Opticell
2	1	Sunflower
3	1	Control
4	1	Malt culm
5	2	Opticell
6	2	Malt culm
7	2	Sunflower
8	2	Control
9	3	Opticell
10	3	Sunflower
11	3	Control
12	3	Malt culm
13	4	Opticell
14	4	Malt culm
15	4	Control
16	4	Sunflower
17	5	Opticell
18	5	Malt culm
19	5	Control
20	5	Sunflower
21	6	Control
22	6	Malt culm
23	6	Opticell
24	6	Sunflower
25	7	Malt culm
26	7	Control
27	7	Opticell

PEN	BLOCK	TREATMENT
49	13	Control
50	13	Sunflower
51	13	Malt culm
52	13	Opticell
53	14	Control
54	14	Sunflower
55	14	Opticell
56	14	Malt culm
57	15	Malt culm
58	15	Sunflower
59	15	Opticell
60	15	Control
61	16	Opticell
62	16	Control
63	16	Malt culm
64	16	Sunflower
65	17	Opticell
66	17	Sunflower
67	17	Control
68	17	Malt culm
69	18	Sunflower
70	18	Control
71	18	Malt culm
72	18	Opticell
73	19	Opticell
74	19	Sunflower
75	19	Malt culm

28	7	Sunflower
29	8	Opticell
30	8	Control
31	8	Malt culm
32	8	Sunflower
33	9	Control
34	9	Malt culm
35	9	Sunflower
36	9	Opticell
37	10	Sunflower
38	10	Control
39	10	Malt culm
40	10	Opticell
41	11	Opticell
42	11	Control
43	11	Sunflower
44	11	Malt culm
45	12	Malt culm
46	12	Sunflower
47	12	Control
48	12	Opticell

76	19	Control
77	20	Malt culm
78	20	Opticell
79	20	Control
80	20	Sunflower
81	21	Opticell
82	21	Control
83	21	Sunflower
84	21	Malt culm
85	22	Malt culm
86	22	Sunflower
87	22	Opticell
88	22	Control
89	23	Malt culm
90	23	Sunflower
91	23	Control
92	23	Opticell
93	24	Control
94	24	Sunflower
95	24	Malt culm
96	24	Opticell

3.3 Data

3.3.1 Production parameters

These parameters were measured every seven days (7, 14, 21 and 28 days of age) and again on the day before slaughter (32 days of age).

All parameters were measured per pen, which equates to 24 repetitions per group.

3.3.1.1 Growth (live body weight and average daily gain)

The birds' growth was assessed by totaling the live weight of all birds in a pen weekly.

Average daily gain (ADG) was calculated with the total weight per pen being divided by the number of birds, and then the number of days in the specified period.

Weights were also recorded for each mortality. This data was not used in the calculations for live body weight or ADG but was used for the calculation of mortality-corrected FCR (described below).

3.3.1.2 Feed intake and feed conversion ratio

The total weight of the feed used per period was measured for each pen.

The mortality-corrected feed conversion ratio (FCR) was calculated by dividing the total feed used per week by the total weekly weight (live & mortalities) gain.

3.3.1.3 Weekly mortality rate

The total amount of birds that died per week, was stated as a percentage of the initial number of birds placed per pen, which was 51 birds.

The mortality rates in the trial were artificially elevated due to the initial selection in week 1 and weekly culling of birds for gut measurements. This equaled one bird being removed from every 2nd pen, each week (12 birds per treatment, each week).

3.3.2 Gut measurements

Birds were sacrificed every seven days, with the parameters discussed below being measured on the gastrointestinal tract during post mortal evaluation. One bird per pen was sacrificed weekly from every 2nd block. The blocks sampled were alternated each week.

3.3.2.1 Gizzard pH

The gizzard pH was measured using a “Hanna HI 98190 pH waterproof portable meter”. Upon culling the gizzard was the first organ to be opened, with the meter’s tip put directly into the gizzard and its contents.

3.3.2.2 Gizzard erosions

Elanco Animal Health’s 2010 Broiler Disease Guide was used to assess the gizzard erosions (Elanco, 2010).

The scoring system can be described as follows:

- Score 0 indicates a normal smooth lining.
- Score 1 indicates roughening of the gizzard lining.
- Score 2 indicates erosions of the gizzard lining.
- Score 3 indicates erosions extending into the mucosal area.

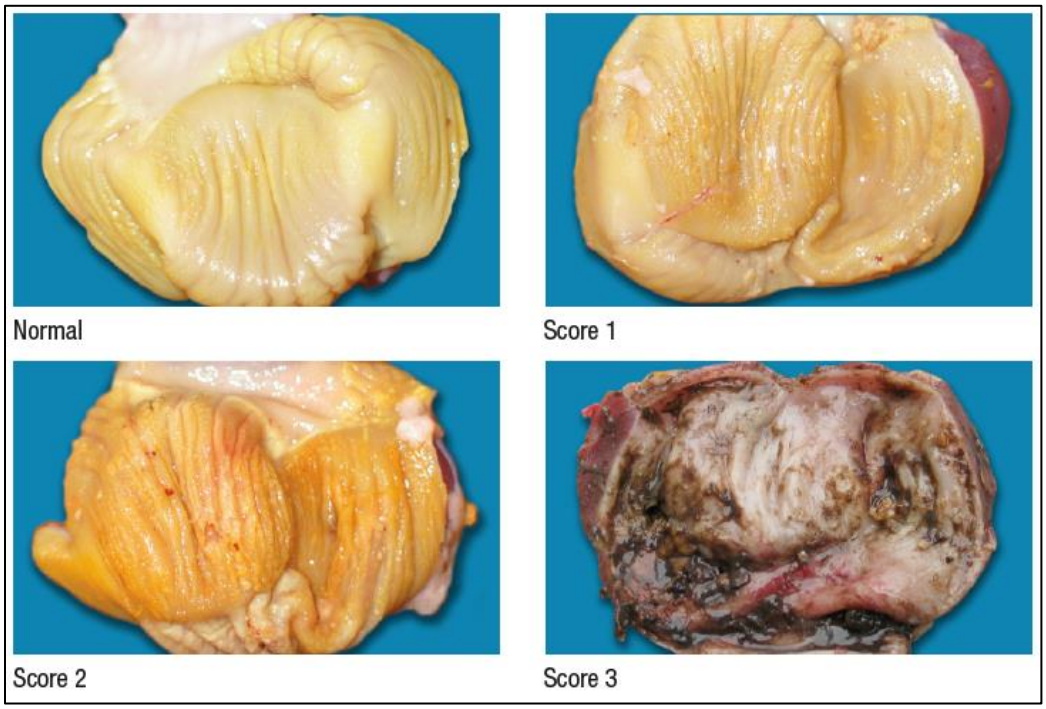

Figure 2: Pictures as example of each gizzard erosion score (Elanco, 2010)

3.3.2.3 Gizzard weights

Gizzards were removed *in toto*, rinsed free from contents, drip dried and then weighed individually.

3.3.2.4 Intestinal lengths

The intestine stretches from the proximal duodenum to the distal colon. The entire length of the intestine was straightened in order to simplify measuring. This included breaking the duodenal loop's attachments to the pancreas. In order to compare the intestinal lengths of different sized birds, the lengths in cm (centimetres) were expressed as ratios to body weights.

3.3.2.5 Caecal lengths

The lengths of both caeca were measured in cm, and the average per bird used for statistical analysis.

3.3.3 Immune system

3.3.3.1 Bursa of Fabricius

Bursa of Fabricius weights were recorded weekly on the same birds sacrificed for the gut measurements. In order to compare the bursas of different sized birds, the bursa weights were expresses as ratios to body weight.

3.3.3.2 Serology

Serology was done by means of BioChek's ELISA kit for NDV, performed at CAL (Central Analytical Laboratories) in Roodepoort, South Africa. Blood samples (2 mL) were collected by venipuncture of the wing vein on the day before slaughter, from 1 bird per pen. This equals 2% of birds being sampled in total, or 24 repetitions per treatment.

3.4 Statistical analysis

Data was recorded manually and transferred to Excel spreadsheets.

Each outcome (live body weight, ADG, etc.) was compared for each of the treatment groups versus the control group at time points (age) using linear mixed models with fixed effects for group, age, block and a group X age interaction, a random effect for pen, and a Bonferroni adjustment for multiple comparisons. The log10-transformed NDV titres were compared between groups using analysis of variance. The significance level was set at 0.05. Statistical analyses were done using Stata 17.0 (StataCorp, College Station, TX, USA).

Comparisons were thus only done between each group and the control, with no between-group comparisons.

CHAPTER 4: RESULTS

Values in tables marked with an asterisk are significantly different from the control. Mean values are indicated in figures. Sunflower hulls are abbreviated as “Sun hulls” in all figures.

4.1 Production parameters

4.1.1 Growth

4.1.1.1 Live body weights per week

No statistically significant differences were observed, but the malt culms group showed numerically lower weights at 21 and 32 days.

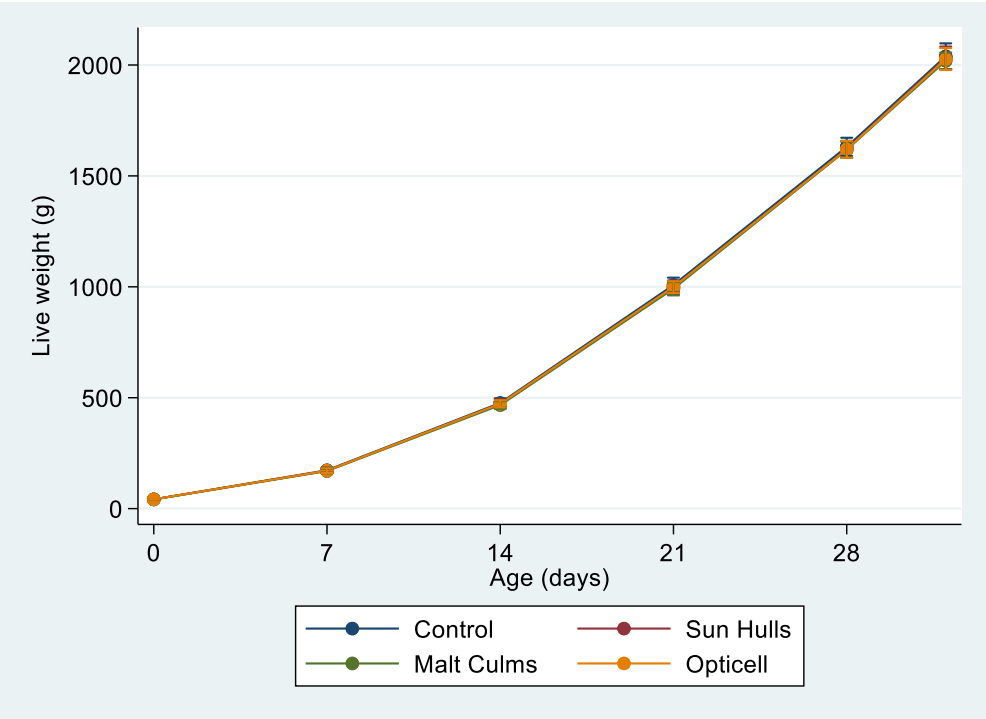

Figure 3: Live body weights per week in chickens fed different diets

4.1.1.2 Average Daily Gain (ADG) per week

No statistically significant differences were observed, but the malt culms group showed a numerically lower ADG for the fifth week.

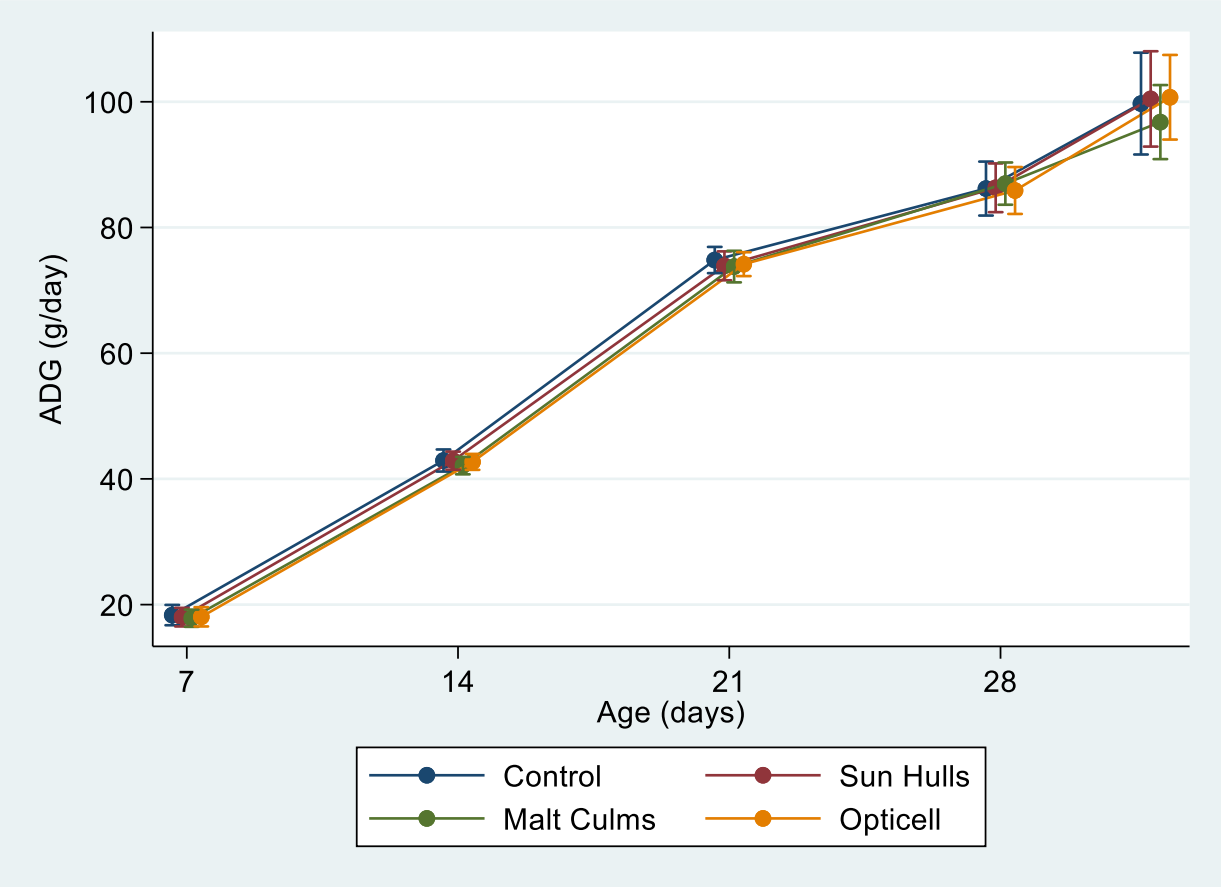

Figure 4: Average Daily Gain (ADG) per week in chickens fed different diets

4.1.2 Feed Conversion Ratio (FCR) per week

No statistically significant differences were observed, but the OptiCell® group showed a numerically higher FCR for the first week.

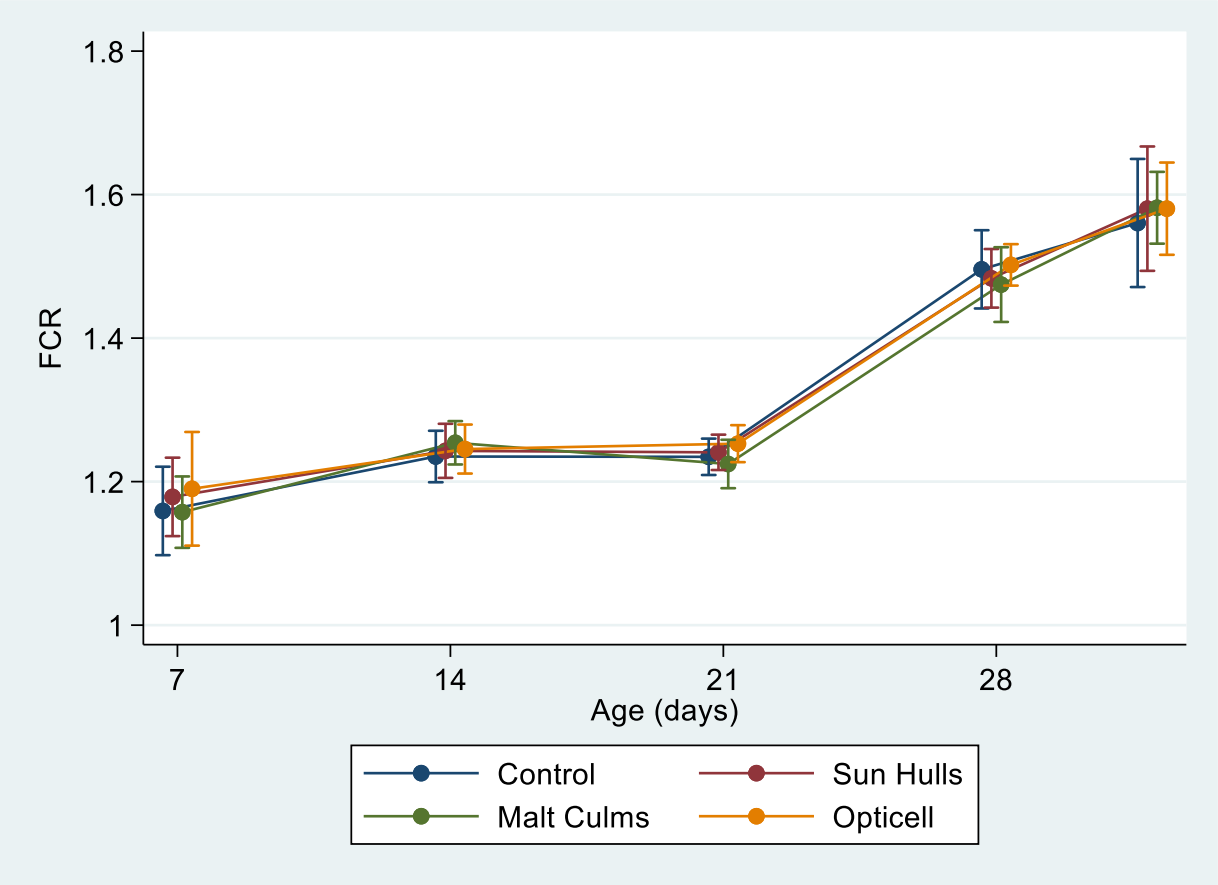

Figure 5: Feed Conversion Ratio (FCR) per week in chickens fed different diets

4.1.3 Weekly mortality %

No statistically significant differences were observed.

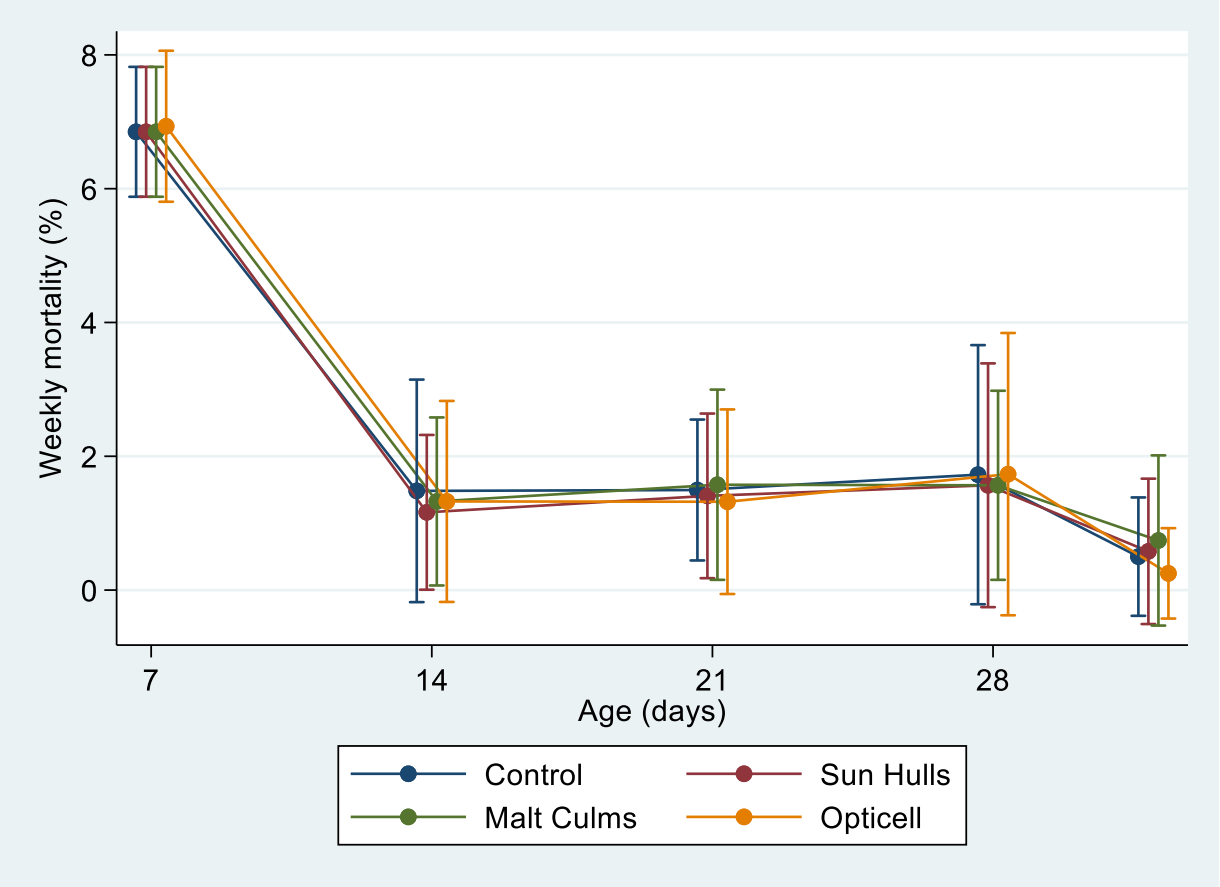

Figure 6: Mortality % per week in chickens fed different diets

4.2 Intestinal health

4.2.1 Gizzard pH

The OptiCell® group had a gizzard pH that was statistically significantly higher than the control group at 28 days ($p = 0.0053$).

The Sunflower hulls group showed a numerically higher pH at 28 days.

Table 6: Mean gizzard pH per week.

	Treatment			
	Control	Sun Hulls	Malt Culms	Opticell
Age (days)				
7	2.56	2.34	2.51	2.43
14	2.27	2.13	2.32	2.23
21	2.54	2.51	2.27	2.29
28	2.43	2.88	2.77	**3.07***

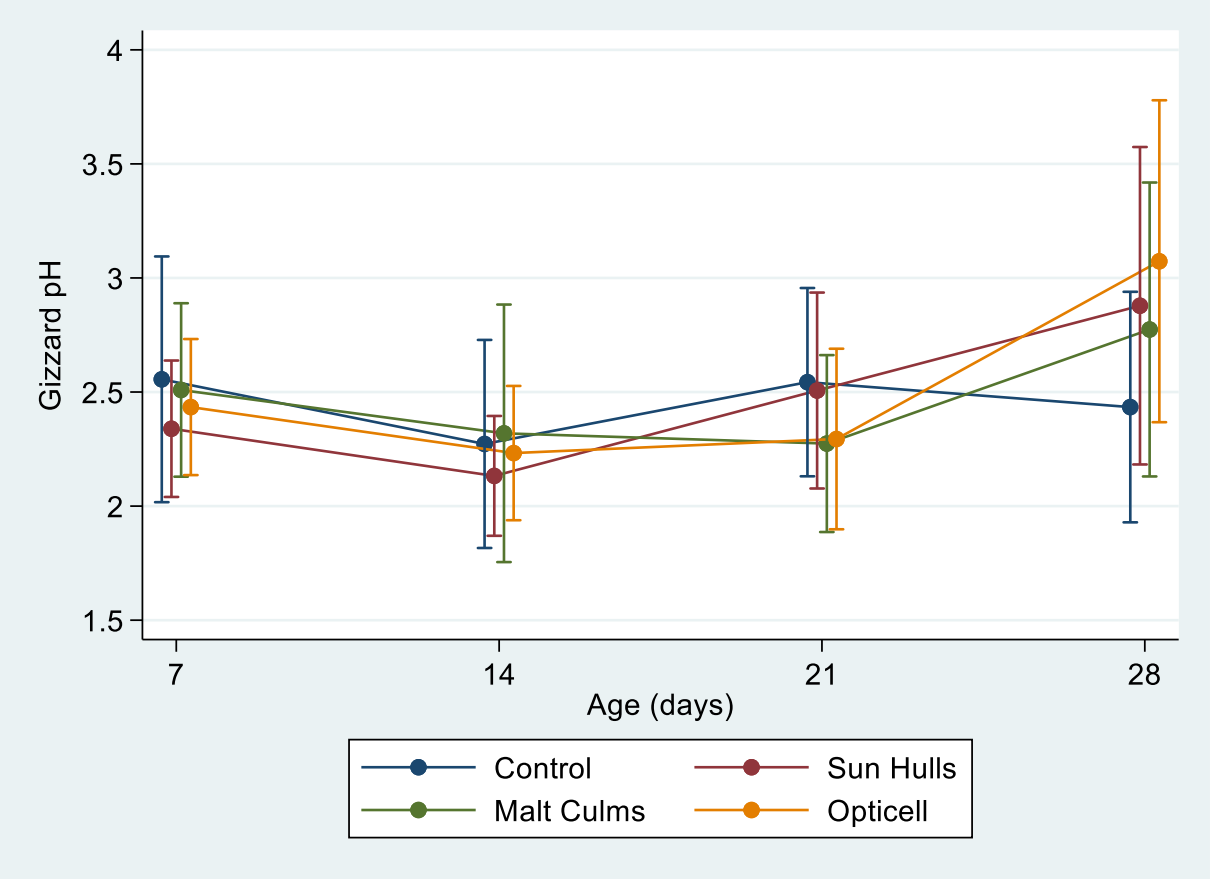

Figure 7: Gizzard pH per week in chickens fed different diets

4.2.2 Gizzard erosion scores

No statistically significant differences were observed.

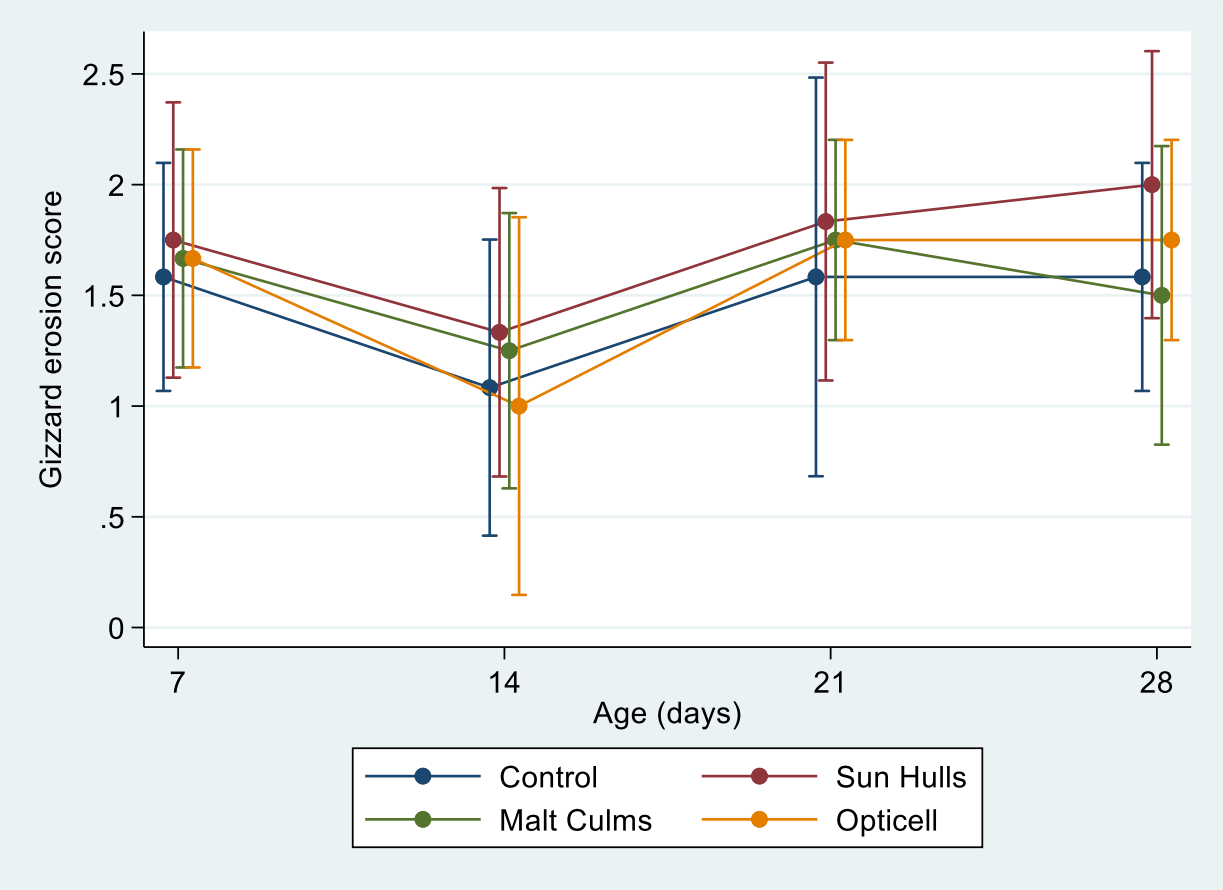

Figure 8: Gizzard erosion score per week in chickens fed different diets

4.2.3 Gizzard weight to body weight ratios

No statistically significant differences were observed, but the malt culms group showed a numerically higher ratio at 7 days.

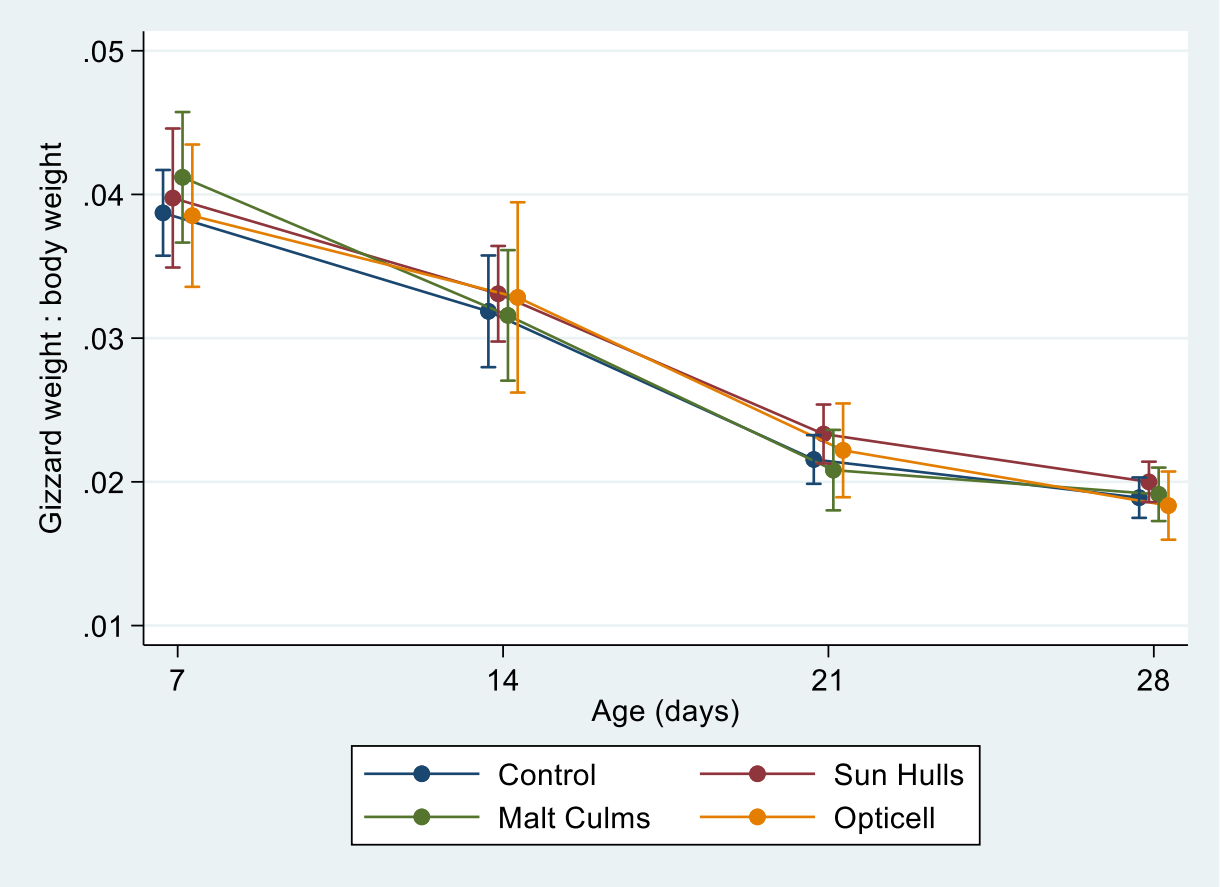

Figure 9: Gizzard weight : Body weight ratios per week in chickens fed different diets

4.2.4 Total intestinal length to body weight ratios

The Sunflower hulls group had a ratio that was statistically significantly higher than the control group at 7 days ($p = 0.02$).

Interesting to note were the ranges of lengths over all treatments for different ages:

- d7, 98 – 132 cm
- d14, 117 – 184 cm
- d21, 155 – 200 cm
- d28, 163 – 219 cm

Table 7: Mean Intestinal length : Body weight ratios per week.

	Treatment			
	Control	Sun Hulls	Malt Culms	Opticell
Age (days)				
7	0.593	**0.662***	0.617	0.611
14	0.326	0.329	0.350	0.351
21	0.166	0.174	0.164	0.169
28	0.114	0.115	0.120	0.114

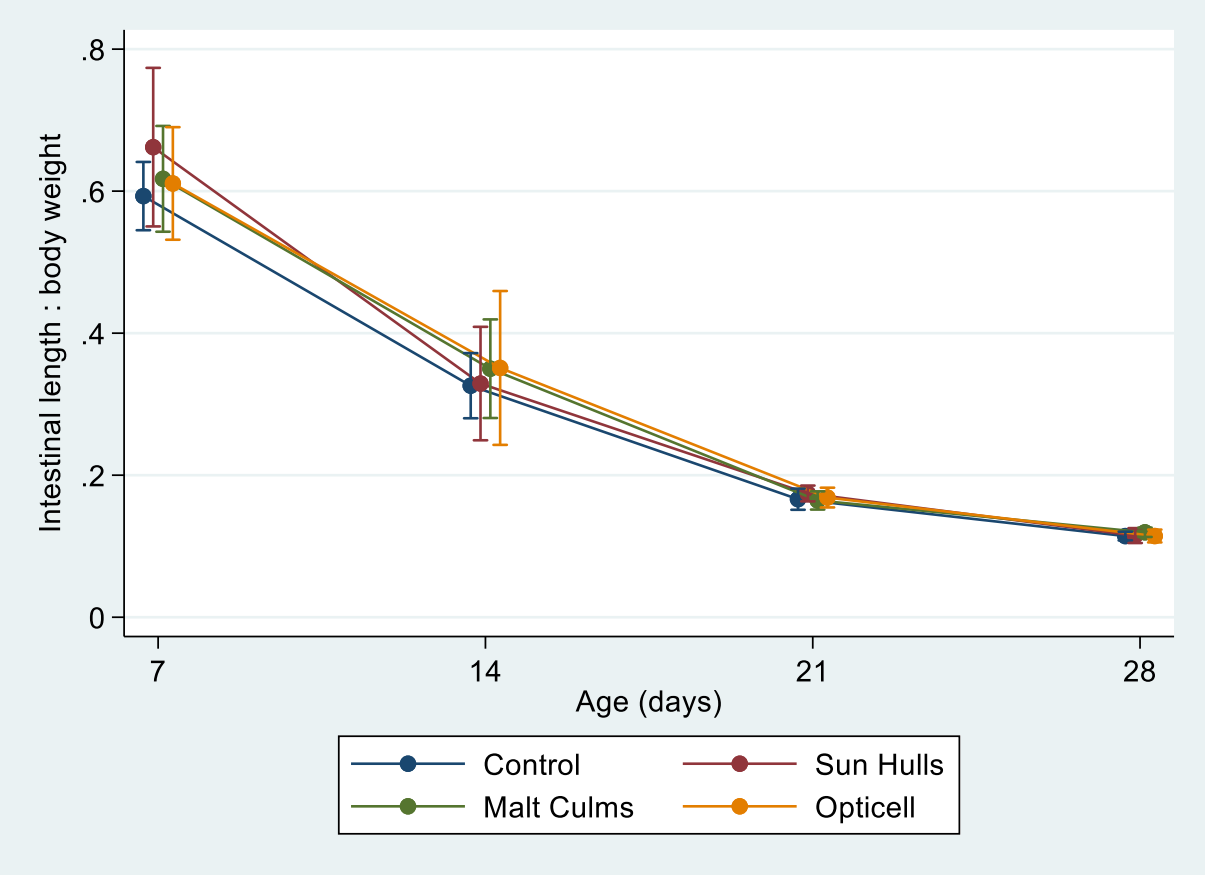

Figure 10: Intestinal length : Body weight ratios per week in chickens fed different diets

4.2.5 Caecal length to body weight ratios

Unsurprisingly, similar to the total intestinal length data, the Sunflower hulls group also had a caecal length ratio that was statistically significantly higher than the control group at 7 days (p = 0.03).

The Malt culms group showed a numerically higher ratio at 14 days.

Interesting to note were the ranges of caecal lengths over all treatments for different ages:

- d7, 8 – 11 cm
- d14, 9 – 14 cm
- d21, 12 – 18 cm
- d28, 14 – 20 cm

Table 8: Mean Ceacal length : Body weight ratios per week.

	Treatment			
	Control	Sun Hulls	Malt Culms	Opticell
Age (days)				
7	0.0458	**0.0516***	0.0457	0.0482
14	0.0248	0.0262	0.0284	0.0270
21	0.0139	0.0141	0.0135	0.0137
28	0.0099	0.0100	0.0101	0.0100

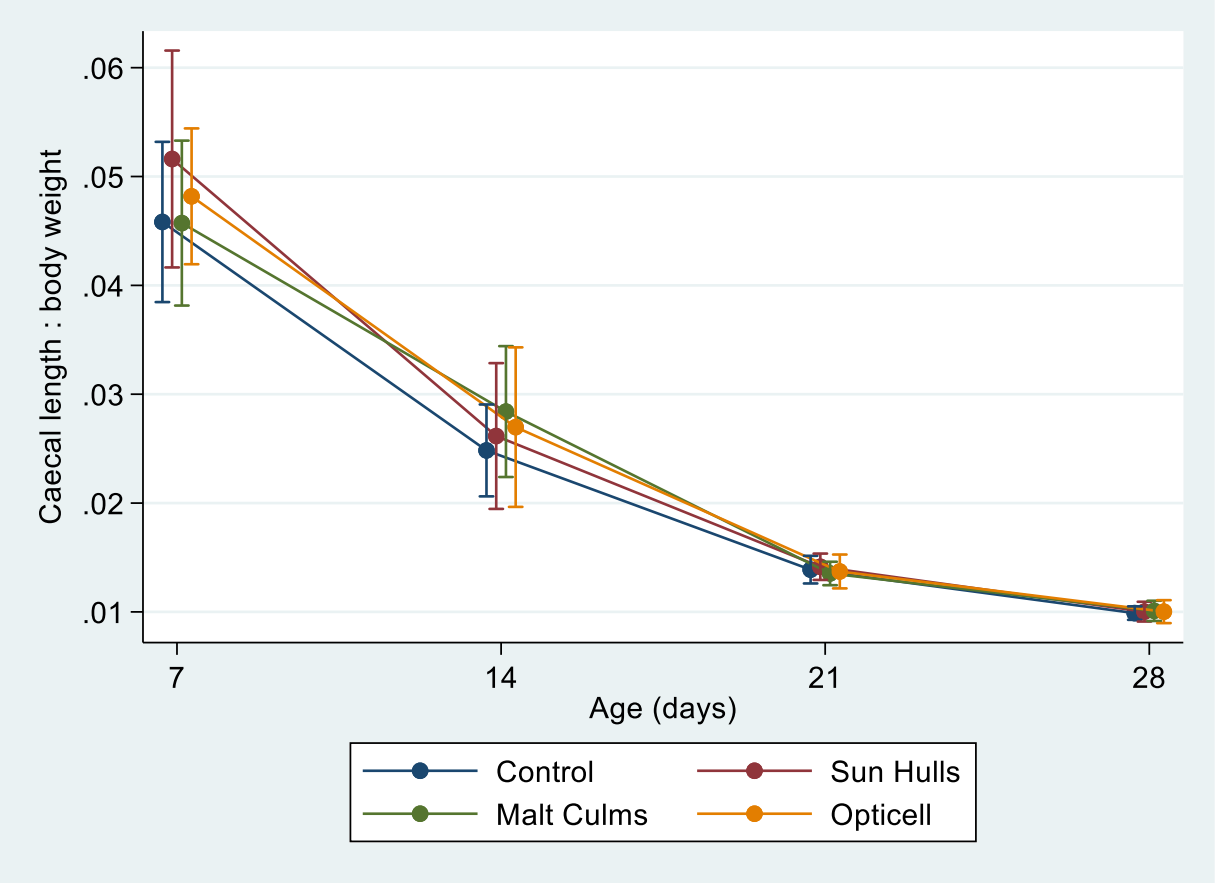

Figure 11: Caecal length : Body weight ratios per week in chickens fed different diets

4.3 Immune system

4.3.1 Bursa weight to body weight ratios

No statistically significant differences were observed.

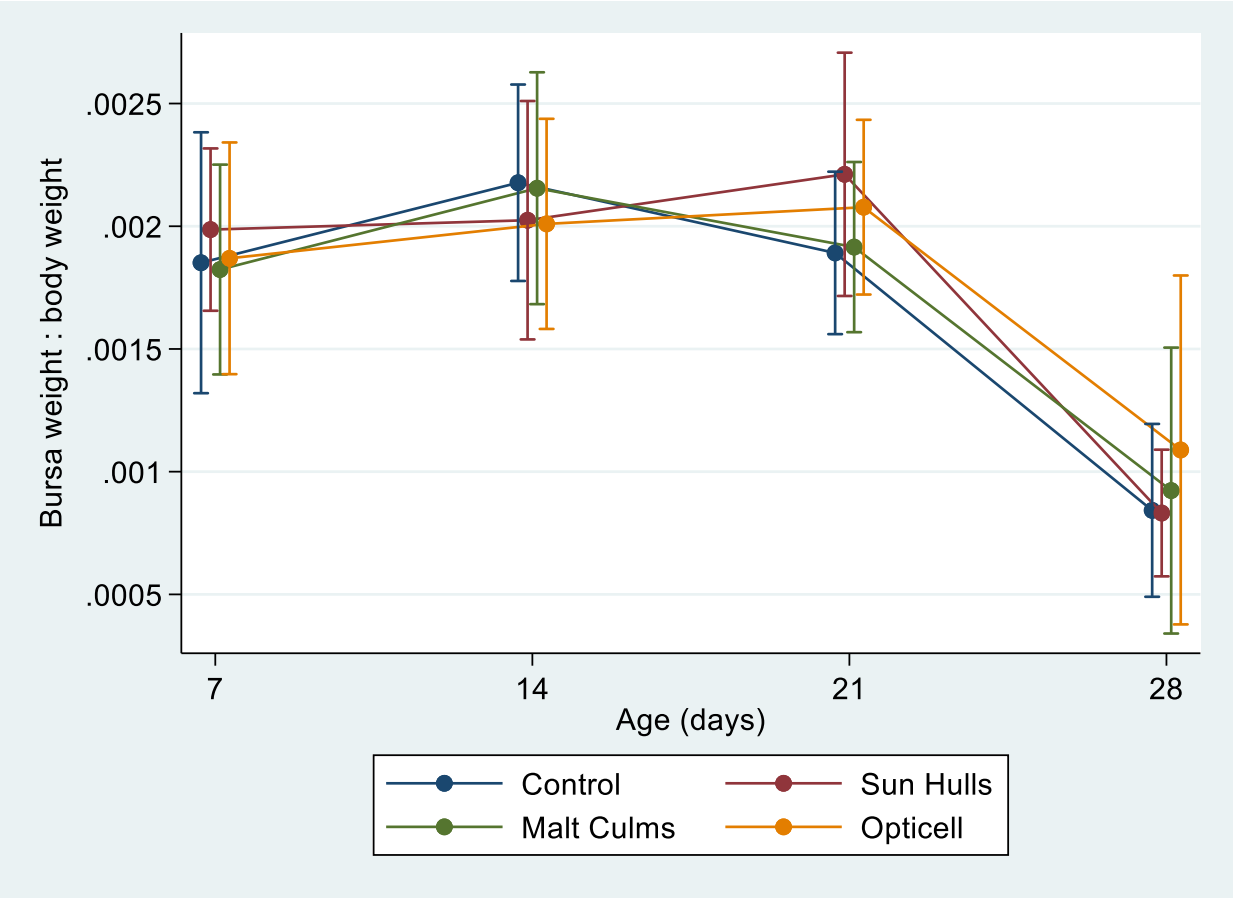

Figure 12: Bursa weight : Body weight ratios per week in chickens fed different diets

4.3.2 Newcastle Disease Virus titres

No statistically significant differences were observed. Outliers are indicated by solid dots on the box plot.

The median titre for the control group was 873.

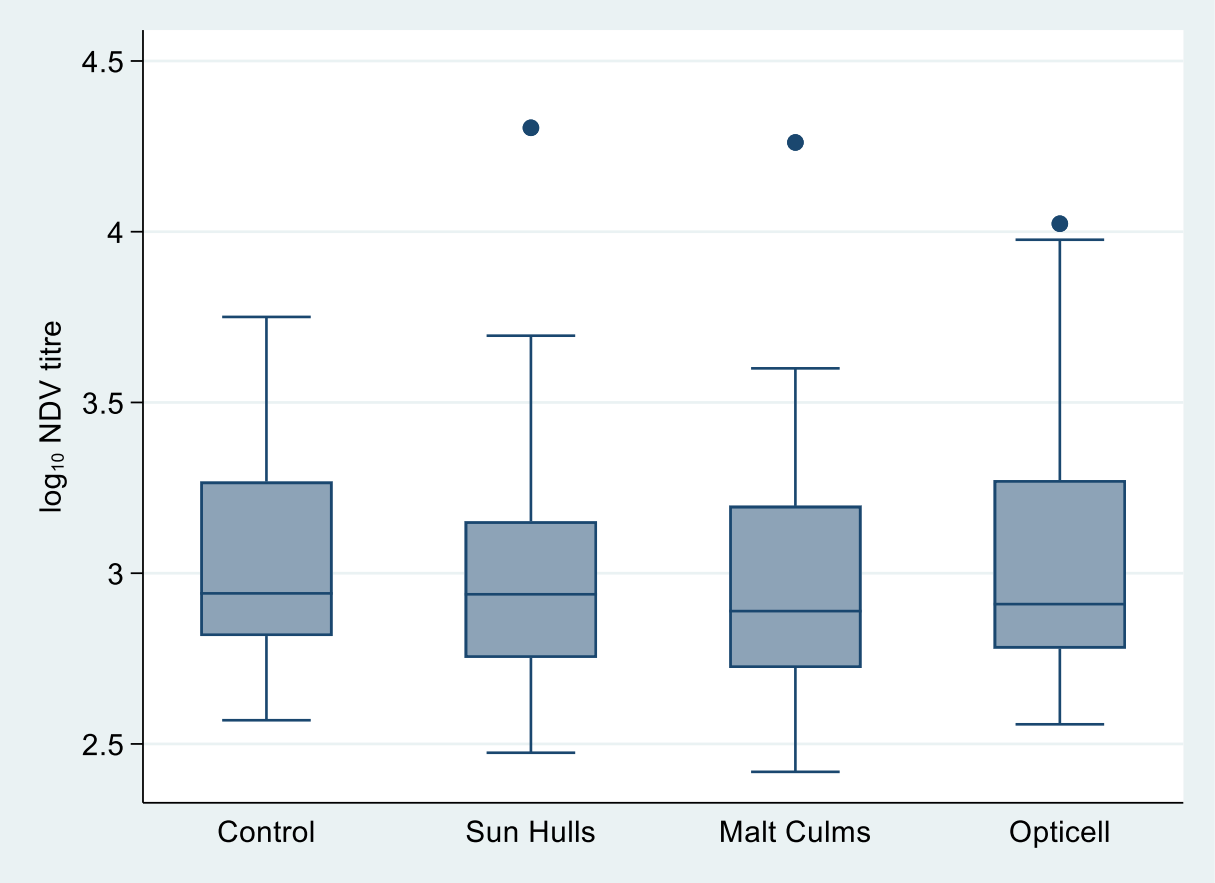

Figure 13: NDV titres per treatment in chickens fed different diets

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1 Discussion

Only two parameters showed statistically significant differences compared to the control group. Firstly, the sunflower hull group had proportionately longer intestines (including the separately measured caecal parameter) at 7 days. Secondly, the OptiCell® group had a higher gizzard pH at 28 days.

The sunflower hull group's longer intestines at 7 days indicates improved early development of the intestinal tract. A better developed intestinal tract should lead to improved feed efficiency. No statistically significant benefits were reflected in the production parameters of this group.

The range of intestinal lengths measured at different ages could serve as a baseline and reference. The lengths seen at 7 days (98 – 132 cm), 14 days (117 – 184 cm), 21 days (155 – 200 cm) and 28 days (163 – 219 cm), compares well with Ross 308 data in 2017 that showed total intestinal lengths of about 210 cm at 42 days of age and a 2 101 g body weight (Kokoszynski et al., 2017). The individual caecal lengths measured at 7 days (8 – 11 cm), 14 days (9 – 14 cm), 21 days (12 – 18 cm) and 28 days (14 – 20 cm) similarly compare well with Kokoszynski's data, showing about 20.5 cm at 42 days of age.

The OptiCell® group had a statistically significant higher gizzard pH at 28 days, with the sunflower hulls group showing a numerically higher pH at the same age. A higher pH did not promote performance. There were wide differences between ages and groups. Gizzard pH of the control group remained relatively constant with means ranging from 2.27 – 2.56 over the 4 measurement periods. This compares with Lee's pH measurements in broilers, ranging from 1.99 – 2.29 (Lee et al., 2021). Nishi (2016), however, reported a pH of 3.51 at 28 days.

Although not statistically significant, the malt culms group showed a numerically higher gizzard to body weight ratio at 7 days, numerically lower body weights at 21 and 32 days, and a lower ADG for the 28 – 32 day period. The crude fibre value of malt culms is 15%, compared with 50% and 59% for sunflower hulls & OptiCell® respectively. This may explain the slight differences observed.

5.2 Conclusion

Previous trials with sunflower hulls and other fibre sources such as oat hulls, sugar beet pulp and rice hulls showed higher gizzard weights, lowered gizzard pH, lowered FCR and improved humoral immunity. These promising responses seen in other trials were not observed in this trial.

Proportional intestinal length is the only new parameter which showed promise, specifically with the sunflower hulls group. Owing to improved early development of the intestinal tract in this group at a 2% inclusion level, there may be merit in including sunflower hulls in the pre-starter period (days 0-7). Longer intestinal tracts did not translate to production advantages here. Different inclusion levels could be trialed. An inclusion level of 1% may be sufficient to show some advantage without changing the base diet much. An inclusion level of 3% may be cost-prohibitive on a commercial scale, due to costly ingredients required to keep the diets isocaloric.

Finally, OptiCell® could be trialed at similar inclusion levels to the sunflower hulls. Its recommended inclusion level of 0.8% may be too low to show significant effects.

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ADDENDUM A: Animal Ethics Committee Approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

23 June 2022

Approval Certificate
New Application

AEC Reference No.:	REC206-21
Title:	The effect of different dietary fibre raw material sources on the production and gut development in fast-growing broilers
Researcher:	Dr AB Fourie
Student's Supervisor:	Dr DBR Wandrag

Dear Dr AB Fourie,

The **New Application** as supported by documents received between 2022-01-25 and 2022-05-30 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2022-05-30.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Number
Poultry - Broilers	4608
Samples	Number
Poultry - Blood Samples from live animals	96

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-06-23.
3. Please remember to use your protocol number (REC206-21) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

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Fakulteit Veeartsenykunde
Lefapha la Disense tša Bongakadiruiwa

ADDENDUM B: Section 20 Approval

agriculture, land reform & rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development
Private Bag X138, Pretoria 0001

Enquiries: Ms Mama Laing • Tel: +27 12 319 7442 • Fax: +27 12 319 7470 • E-mail: MamaL@dalrrd.gov.za
Reference: 12/11/1/4/5 (2407BD)

Responsible person(s): Dr Andries Benjamin Fourie
Institution: Grain Field Chickens Workers' Trust Trial Pens
Email: driesfour@gmail.com

Dear Dr Fourie,

CONDITIONS FOR RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984)

Title of research project / study: "The effect of different dietary fibre raw material sources on the production and gut development in fast-growing broilers"

Your application, requesting permission under Section 20 of the Animal Diseases Act, 1984 (Act no 35 of 1984) to perform the research project or study stipulated above, refers.

1. Based on the information provided in your application, the Director of Animal Health has no objection to this study. The study may continue if statement 1.1 to 1.6 hereunder are, and remain, accurate. **Should the scope of your research project change in any way you are required to inform the Section 20 Secretariat and may not proceed with any activities until written permission to do so have been granted by the National Director: Animal Health.**

   1.1. No work will be done with controlled and notifiable animal diseases (list can be obtained / requested from this office), which includes any animal diseases which do not occur in South Africa;
   1.2. No imported material of animal origin or imported animal pathogens will be utilized in the study;

1.3. No samples that originate from a biobank will be used in the study;
1.4. No clinical studies will be performed in the target species, either in a laboratory or in the field;
1.5. The areas where the samples are to be collected are not under restriction for controlled or notifiable diseases to which the species of animal, from which the samples are obtained, is susceptible;
1.6. No samples or products that have not been passed as fit for human consumption will be obtained from an abattoir.

2. In addition to the conditions mentioned in point 1, you are responsible for ensuring that your research project or study complies with all or part of the following, as applicable:

2.1. Permission to perform research under Section 20 of the Animal Diseases Act 1984 (Act no 35 of 1984) does not relieve the researcher of any responsibility which may be placed on him/her by any other Act of the Republic of South Africa, including the Veterinary and Para-Veterinary Professions Act 1982 (Act No. 19 of 82), the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 1947 (Act no 36 of 47), the Medicines and Related Substances Control Act 1965 (Act 101 of 65), the Genetically Modified Organisms Act, 1997 (Act No 15 of 1997) and the National Environmental Management: Biodiversity Act, 2004 (Act No 10 of 2004);
2.2. No part of the study may begin until valid ethical approval has been obtained in writing from the relevant South African authority;
2.3. Any incidence or suspected incidence of a controlled or notifiable disease in terms of the Animal Diseases Act 1984 (Act no 35 of 1984), must be reported immediately to the responsible state veterinarian;
2.4. Four thousand, six hundred and eight chickens originating from Eagle's Pride Hatcheries and to be housed on Grain Field Chickens Workers' Trust Trial Farm, for which a state veterinary letters were obtained, may be used for this study;
2.5. The blood samples may be analysed in the Central Analytical Laboratories, Stormill, Roodepoort;
2.6. Samples or material may not be outsourced or used for further/other research without prior written approval from the Director of Animal Health.
2.7. All carcass material after dissection at Grain Field Chickens Workers' Trust Trial Farm is to be considered condemned in terms of the Meat Safety Act

2000 (Act No 40 of 2000) and must be disposed of through compost or incineration at a registered abattoir

2.8. All potentially infectious material utilised or generated during or by the study is to be destroyed at completion of the study and only a registered waste disposal company may be used for the removal of waste generated during or by the study;

2.9. Records must be kept for five years for auditing purposes.

Written permission from the Director of Animal Health must be obtained prior to any deviation from the conditions. Application must be sent in writing to MarnaL@dalrrd.gov.za.

Failure to obtain written permission as above may be considered a contravention of the Animal Diseases Act, 1984 (Act no 35 of 1984).

Expiry date of this permit: 31 June 2025

Kind regards,

DR. MPHO MAJA
DIRECTOR: ANIMAL HEALTH
Date: 2022 -06- 20