The effect of Nutrifen® and Nutrifen Plus® in the diet of Hy-Line layers on production, egg quality and egg shelf life

by

Chericke Ann Williams

Thesis presented in fulfilment of the requirements for the degree of

Master of Science in Agriculture (Animal Sciences)

at

Stellenbosch University

Supervisor: Dr Elsje Pieterse

Department of Animal Science, Faculty of AgriSciences

April 2019

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: April 2019

Summary

The use of natural feed additives for animal production has become an increasing topic of interest due to the ban of antibiotics in some countries and the shift towards more sustainable and ethical production practices. Fenugreek is a promising natural additive because it has been tested in numerous animal diets (mostly ruminants) and shows improved growth and milk production in animals, together with a decrease in the amount of greenhouse gas emissions. Nutrifen® and Nutrifen Plus® are products derived from the fenugreek plant seeds and used as a natural feed additive. Although fenugreek's effects on layer hen production is understudied, some signs of improvement in egg production and egg quality have been reported. This study was conducted to evaluate the effect of Nutrifen® and Nutrifen Plus® on the production, quality and shelf life of Hy-Line layer hen eggs. The effects of five different diets (treatments) were explored: control, Nutrifen® 0.1% (N1), Nutrifen® 0.2% (N2), Nutrifen® Plus® 0.1% (N+1) and Nutrifen Plus® 0.2% (N+2). All the treatments consisted of the control with an addition (a percentage as indicated) of Nutrifen® or Nutrifen Plus®.

The first part of the experiment determined the total intake, number of eggs produced, egg weight, energy intake, lysine intake, protein intake, body weight and feed conversion ratio (FCR) of the layers. The correlation between intake, energy intake, lysine intake and protein intake with respects to egg production was also calculated. The diets were fed to the layer hens for one month and the results recorded showed no significant differences between the Nutrifen® and Nutrifen Plus® treatments compared to the control for all the parameters evaluated. However, only small tendencies to differ were observed between N1, N+1 and N2 regarding the total intake, lysine intake and energy intake.

In the second part of the experiment, egg quality parameters were tested after one month in storage and again after three months in storage. The egg quality parameters consisted of whole egg, egg shell, egg yolk and egg white quality. The shelf life was tested through the storage of the eggs in a laboratory room at 15 to 18°C for three months (90 days) after collection day, after which a quality evaluation test was performed. Egg quality analysis was performed on half (15) of the eggs on day 30 after collection while the other half of the eggs were stored for 90 days and analysed using the same egg quality analysis.

No significant differences (P >0.05) between treatments were observed for all the respective egg quality parameters in the first analysis (one month), except for yolk colour L*. Nutrifen® 0.2% showed a significant (P ≤0.05) increase in the colour L* value of the egg yolk compared to the other treatments, which results in eggs with a whiter appearance. No significant differences were observed among the other treatments regarding the egg yolk lightness (colour L*) values.

No positive or negative differences between treatments at month three were observed for most of the egg quality parameters measured except for the colour L* and colour b* values of the yolk. Treatment Nutrifen® 0.2% resulted in whiter egg yolks compared to the control and all other treatments due to a higher colour L* value measured ($P \leq 0.05$). In addition, treatment Nutrifen® 0.1% had a more yellow colour compared to the control and treatments Nutrifen® 0.2% and Nutrifen Plus® 0.2% due to a higher colour b* value measured ($P \leq 0.05$).

Significant differences ($P \leq 0.05$) were also observed before and after storage. A decline in the egg weight, albumen weight, yolk height and albumen height were evident between treatments from month one to month three in storage. An increase in the yolk weight was also observed during storage of eggs after three months. In addition, storage also affected the egg yolk colour, with an increase in the colour L* value (whiteness) of the egg for all treatments after being stored for three months. An increased ability of treatment Nutrifen® 0.1% to maintain its yellow colour (higher colour b* value) was observed compared to the control and treatments Nutrifen® 0.2% and Nutrifen Plus® 0.2%.

The overall evidence suggests that more research is needed to further investigate the effect of Nutrifen® and Nutrifen Plus® on production and egg quality parameters. The results obtained from this study show the potential of Nutrifen® and Nutrifen Plus® products to improve production in terms of reducing the effects of long-term storage on egg weight and yolk colour. The yolk colour was largely affected by the treatment diets. Therefore, a more in-depth investigation into the effect of the treatment diets on yolk colour is necessary, as yolk colour is important for consumers.

Opsomming

Die gebruik van natuurlike bymiddels vir diereproduksie het 'n belangrike onderwerp geword vanweë die verbod op antibiotika in sommige lande, asook die verskuiwing na meer volhoubare en etiese produksiepraktyke. Fenegriek is 'n belowende natuurlike bymiddel, aangesien dit al in verskeie diere se diëte (meestal herkouers) getoets is en voordelige resultate gelewer het. Dié resultate sluit ondermeer in verbeterde groei en melkproduksie, asook ’n afname in kweekhuisgas vrystellings in. Nutrifen® en Nutrifen Plus® is produkte wat gemaak word van fenegriek plantsaad en word gebruik as 'n natuurlike bymiddel. Daar is tans nog nie baie navorsing gedoen oor die effek van fenegriek op die produksie van lêhenne nie, maar tekens van verbetering in eierproduksie en eiergehalte is al gerapporteer. Die doel van hierdie studie was om die effek van Nutrifen® en Nutrifen Plus® op die produksie, gehalte en rakleeftyd van Hy-Line lêhenne se eiers te evalueer. Die effek van vyf verskillende diëte (behandelings) is ondersoek: ‘n Kontrole, Nutrifen® 0.1% (N1), Nutrifen® 0.2% (N2), Nutrifen® Plus® 0.1% (N+1) en Nutrifen Plus® 0.2% (N+2). Die verskillende behandelings het bestaan uit die kontrole en ‘n basisdieet. Die basisdieet is onderskeidelik vervang met ‘n persentasie (soos aangedui) van Nutrifen® en Nutrifen Plus®.

In die eerste deel van die eksperiment is die totale inname, aantal eiers geproduseer, eiergewig, energie inname, lisien inname, proteïen inname, liggaamsgewig en voeromsetverhouding van die lêhenne bepaal. Die korrelasie tussen inname, energie inname, lisien inname en proteïen inname met betrekking tot eierproduksie is ook bereken. Die lêhenne is vir een maand met die diëte gevoer. Vir elk van die parameters gemeet is daar egter geen betekenisvolle verskille tussen die Nutrifen® en Nutrifen Plus® behandelings in vergelyking met die kontrole gevind nie. Ten opsigte van die totale inname, lisien inname en energie inname was daar egter klein neigings om te verskil waargeneem tussen Nutrifen® 0.1%, Nutrifen Plus® 0.1% en Nutrifen Plus® 0.2%.

In die tweede deel van die eksperiment is eiergehalte op een maand en op drie maande getoets. Die verskille tussen die maande is ook geëvalueer om die rakleeftyd te bepaal. Die eiergehalte parameters het bestaan uit heel eier, eierdop, eiergeel en eierwitgehalte. Die rakleeftyd van die eiers is oor ‘n tydperk van drie maande getoets, waartydens die eiers in 'n koel kamer geberg was. Na die drie-maand periode is 'n eiergehalte evalueringstoets uitgevoer. Die eiergehalte analise is binne een maand op die helfte van die eiers uitgevoer, terwyl die ander helfte van die eiers eers vir drie maande gestoor is en toe dieselfde eiergehalte analise ondergaan het. Daar is geen beduidende verskil ($P > 0.05$) gevind tussen die behandelings vir die onderskeie eiergehalteparameters in die eerste analise (een maand), nie, met die uitsondering van eiergeel kleur L. In vergelyking met die ander behandelings, het Nutrifen Plus® 0.1% 'n beduidende ($P \leq 0.05$) verbetering in die kleur L waarde van die eiergeel getoon. Geen beduidende verskille is waargeneem tussen die ander behandelings ten opsigte van die kleur L waarde nie.

Geen beduidende verskille is gevind tussen die eerste eier analise en die tweede eier analise vir al die parameters ontleed vir elk van die behandelings nie, met die uitsondering van eiergeel kleur L, eiergeel hoogte, eiergeel kleur waaier waarde en dik wit verspreiding. Vir al die behandelings het die analise van eiergeel kleur L op maand een hoër ($P > 0.05$) waardes gehad in vergelyking met die analises van maand drie. Die dik wit verspreiding het ook beduidende agteruitgang vanaf die eerste tot die derde maand getoon, op 'n 5% noemenswaardige vlak. Die eiergeel hoogte en kleur waaier waardes was beduidend laer vir al die behandelings van maand een tot maand drie.

Die resultate van die rakleeftyd studie het beduidende verskille ($P \leq 0.05$) in die eiergeel kleur waaier waardes van die verskillende behandelings getoon. Die kontrole het 'n aansienlike laer kleur waaier waarde in vergelyking met die ander behandelings (wat nie betekenisvol van mekaar verskil het nie) gehad.

Die effek van behandeling op die eierproduksie, eiergehalte en eier rakleeftyd in hierdie studie lewer nie genoeg bewyse om te bevestig dat die gebruik van Nutrifen® en Nutrifen Plus® die algehele produktiwiteit van Hy-Line lêhenne verbeter nie.

Acknowledgements

I would like to express my sincere gratitude to the following people for their contribution and support:

- God, my Heavenly Father, for carrying me through and helping me through what has proven to be a very challenging three years.
- My family, especially my dad, Richard, mom, Cheryl-Ann, and brother, Richard Jnr, for all your love and support throughout this process. My family was always available, and I could count on them for help.
- All the family and friends who dedicated their time to help me with physical work at the farm and lab, and for their support at home during the writing process.
- A very special thank you to Dr Elsje Pieterse, who believed in me and supported me throughout this process. Thank you for the support and guidance and for being a true role model and mentor in such a crucial time of my life.
- The technical team and lab assistants at the Department of Animal Science at Stellenbosch University for your contributions.
- Sarah Erasmus for her assistance with my writing skills.
- Joy Waddell for her assistance with my writing skills.
- Professor Martin Kidd for his assistance with my data analysis.
- My fellow students and friends for always being available and willing to help me, and for the support structure you provided to me.
- Dianca Du Plessis, my partner in crime, for making everything more bearable and for being there when I needed someone.
- Ryan Petersen, for all his support and help when I needed it the most.

Notes

The language and style used in this thesis is in accordance with the *South African Journal of Animal Science,* with changes to increase readability. This thesis represents a compilation of manuscripts, where each chapter is an individual entity; thus, some repetition between chapters has been unavoidable.

Table of Contents

Table of Figures

Table of Tables

Table of Equations

Abbreviations

ADFI	Average daily feed intake
AOAC	Association of Official Analytical Chemists
AWG	Average weight gain
BW	Body weight
CF	Crude fat
CO_2	Carbon dioxide
CP	Crude protein
Cu	Copper
DAFF	Department of Agriculture, Forestry and Fisheries
DM	Dry matter
EE	Ether extract
FCR	Feed conversion ratio
GHs	Growth hormones
H_2SO_4	Sulphuric acid
HU	Haugh Unit
LDLs	Lipoproteins
LSD	Least significant difference
Mn	Manganese
MSM	Methylsulfonylmethane
N	Nitrogen
NaOH	Sodium hydroxide
N1	Nutrifen® 0.1%
N2	Nutrifen® 0.2%
N+1	Nutrifen Plus® 0.1%
N+2	Nutrifen Plus® 0.2%
RYCF	Yolk colour fan scale
SAPA	South African Poultry Association
Se	Selenium
SPBEs	Saw Palmetto berries
TMT	Treatment
ZAR	South African Rand
Zn	Zinc

Chapter 1: General Introduction

1.1 Introduction

The availability, affordability and nutritional composition (e.g., high in protein, essential vitamins, minerals and low in calories) of eggs makes it a popular food source worldwide. The South African Poultry Association (SAPA, 2017) reports that poultry and poultry products, which includes eggs, are the most affordable source of protein. Therefore, it can also be regarded as a valuable food commodity to feed the world's growing population and thus contribute towards food security. As a result, it is vital that the layer hen industry continues to grow and improve.

There is, however, numerous obstacles that threaten and limit the growth of the layer hen industry. One of the major obstacles facing this industry is high feed costs, with feed being the largest expense for a layer hen farmer. Other difficulties for the layer hen industry include the outbreak of diseases (Windhorst, 2006), the use of antibiotics in animal feed and animal welfare issues (Hester, 2005). In recent years, consumers have also greatly impacted on the layer hen industry with demands of improved food safety, animal welfare and environmental conservation (Penz & Bruno, 2011). Consumers are also concerned with the quality of eggs as it influences their purchasing potential. The egg quality is also important for egg producers as it can negatively affect the egg grade and thus the egg price. Therefore, improving egg quality is imperative for the egg industry. The above mentioned problems need to be addressed with economic and sustainable solutions to ensure continuous production for the layer hen industry.

The use of natural feed additives has gained increased interest in the animal feed industry and may offer potential solutions to some of the problems facing the layer hen industry. The use of natural feed additives is also a more sustainable and environmentally friendly method for improving animal production efficiency. Feed additives are commonly used in poultry to improve growth, egg production and hen health. In the past, antimicrobial growth promoters were the most commonly used feed additives. However, due to a recent ban on the use of antibiotics in animal production, alternative products are being explored (Demir *et al.*, 2005). One potential downside of the use of feed additives is that their inclusion may influence (i.e., increase) the price of feed. It is therefore important to quantify the benefits of using a feed additive and to ensure that the benefits outweigh the costs.

Fenugreek is a promising animal feed additive that has been reported to improve growth rate (Hossain *et al.*, 2015) and milk production in ruminants (Tomar *et al.*, 1996). It is reported to increase palatability of feed (Meghwal & Goswami, 2012) and therefore feed intake (Petit *et al.*, 1993). Numerous studies have also reported positive effects of fenugreek on layer hen production (Dankook University, 2013; Abdouli *et al.*, 2014; Motamedi & Talkimi, 2014) and egg quality (Safaa, 2007; Motamedi & Talkimi, 2014; Panaite *et al.*, 2014). For these reasons, it may prove to be valuable to test the effects of fenugreek as a feed additive in the diet of layer hens. Hence, for the purposes of this study, Nutrifen® and Nutrifen Plus® (made from fenugreek seeds) were used as a natural feed additive in the diets of Hy-Line layer hens. The testing of natural feed additives is important for future production as it has the potential to improve sustainability and production, which in turn will lead to growth in the layer hen industry, improved profitability and an increase in the supply of eggs.

1.2 References

Abdouli, H., Haj-Ayed, S., Belhouane, S. & Hcini-Emna, E., 2014. Effect of feeding hens with fenugreek seeds on laying performance, egg quality characteristics, serum and egg yolk cholesterol. J. New. Sci. 1-11.

Dankook University, 2013. Effects of Nutrifen on egg production, quality, nutrient digestibility, blood profiles, fecal microflora, and fecal noxious gas emission in laying hens. Department of Animal Resource and Science, Dankook University, Republic of Korea.

Demir, E., Sarica, Ş., Özcan, M.A. & Suiçmez, M., 2005. The use of natural feed additives as alternative to an antibiotic growth promoter in broiler diets. Arch. Geflügelk. 69(3), 110-116.

Hester, P.Y., 2005. Impact of science and management on the welfare of egg laying strains of hens. Poult. Sci. 84(5), 687-696.

Hossain, M.M., Begum, M., Nyachoti, C.M., Hancock, J.D. & Kim, I.H., 2015. Dietary fenugreek seed extract improves performance and reduces fecal *E. coli* counts and fecal gas emission in lactating sows and suckling piglets. Can. J. Anim. Sci. 95(4), 561-568.

Meghwal, M. & Goswami, T.K., 2012. A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. J. Food. Process. Technol. 3(9), 1-10.

Motamedi, S.M. & Talkimi, M.M., 2014. Investigating the effect of fenugreek seed powder and garlic powder in the diet on immune response of commercial laying hens' egg. Indian J. Sci. Res. 3(1), 277-283.

Panaite, T., Cornescu, M.G. & Criste, R., 2014. Effect of fenugreek supplements to high fatty acids diets of layer performance. Lucr. Ştiinţifice - Ser. Zooteh. 62, 158-163.

Penz, A. & Bruno, D., 2011. The poultry site. Available at: http://www.thepoultrysite.com/articles/2018/challenges-facing-the-global-poultry-industry-to-2020/ [Accessed 03.12.17].

Petit, P., Sauvaire, Y., Ponsin, G., Manteghetti, M., Fave, A. & Ribes, G., 1993. Effects of a fenugreek seed extract on feeding behaviour in the rat: Metabolic-endocrine correlates. Pharmacol. Biochem. Behav. 45, 369-374.

Safaa, H., 2007. Effects of dietary garlic on cholesterol metabolism in laying hens. Egypt. Poult. Sci. 27(IV), 1207-1221.

South African Poultry Association (SAPA), 2017. Key market signals in the egg industry for the second quarter of 2017. 2Q. Available at: http://www.sapoultry.co.za/home/quarterly-reports.php [Accessed 22.11.2018].

Tomar, K., Singh, V. & Yadav, R., 1996. Effect of feeding maithy (*Trigonella foenum-graecum*) and chandrasoor (*Lepidium sativum L.*) seeds on milk and blood constituents of Murrah buffaloes. Indian. J. Anim. Sci. 66(11), 1192-1193.

Windhorst, H.-W., 2006. Changes in poultry production and trade worldwide. Worlds. Poult. Sci. J. 62(4), 585-602.

Chapter 2: Literature Review

2.1 Introduction

Table eggs are one of the most affordable animal protein sources available for human consumption (SAPA, 2017). This, together with the rapid rise in the world's population, has caused the demand for eggs to increase. Extensive research is being done on increasing egg production and egg quality characteristics through feeding mechanisms and breeding (Travel & Nys, 2011). Genetic selection has a significant impact on egg production pre-production, while during the growing and production stage, nutrition is one of the major methods of improving egg production and egg quality. Egg production and quality is however also affected by other factors such as hen age, body condition, environmental stress and hen health. Furthermore, egg quality can also be affected by egg storage time and conditions. All these factors play a vital role in determining egg production and quality.

2.2 Egg production and egg quality

Egg production (number of eggs and egg weight) is of utmost importance in the layer industry and should therefore be optimised for a farm to be profitable. Egg production in layer hens is highly heritable, therefore layers are being genetically altered through breeding them for extended laying periods and shorter open days (i.e., larger clutches within the laying cycle) (Travel & Nys, 2011), which will allow for increased egg production. Genetic modification may result in positive effects such as double yoked eggs, as well as negative effects such as having small and soft-shelled eggs. Each breed of layer hens however differs in terms of production.

A typical egg production and egg weight curve of a layer hen flock for the duration of the laying period (weeks) is presented in Figure 2.1. Peak production above 90% is reached at approximately 6-8 weeks after the start of lay. After peak production is reached, egg production gradually declines (Jacob *et al.*, 2003). The egg weight increases sharply at the start of lay and thereafter gradually increases with age, reaching egg weights greater than 60 g by 12 months of age.

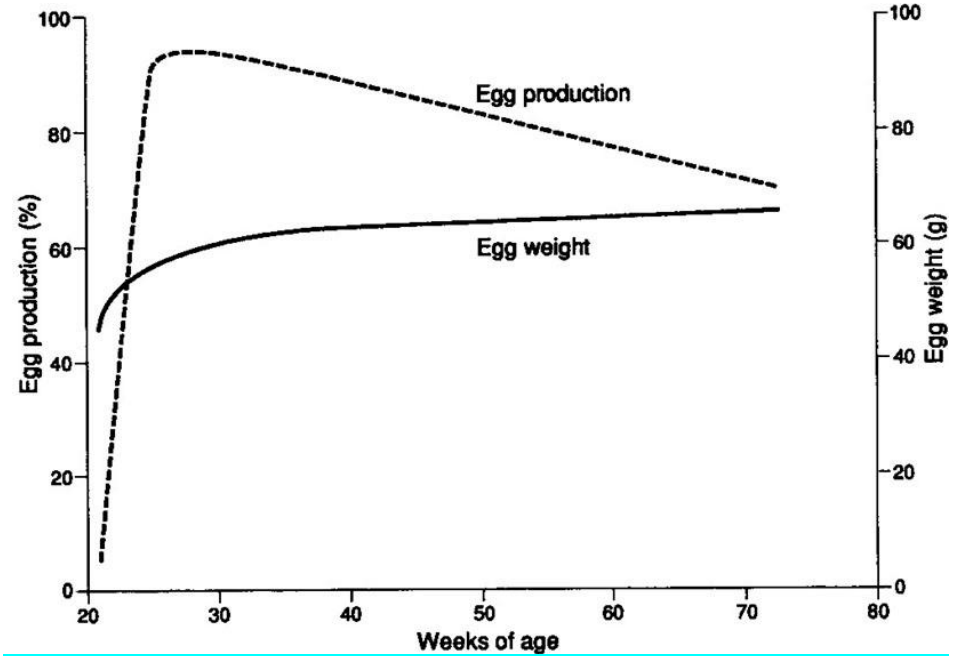

Figure 2.1 The production curve for a typical laying flock (Jacob *et al.*, 2013)

During the complex process of egg production, many other errors may also occur, resulting in egg deformities. Early induced sexual maturity for example may result in small eggs, while soft shells are caused by the early ovulation of a new egg while the other egg is still forming (Travel & Nys, 2011). These deformities will ultimately lead to the downgrading of egg quality (Roberts, 2004).

The South African egg industry loses millions of Rands (ZAR) due to poor egg quality (Roberts, 2004). Eggs are sold and exported commercially as shelled eggs and egg products (powdered and liquid eggs). The internal and external egg quality is therefore of high economic value for the farmer and the consumer. Maintaining a high standard of egg quality is therefore important in order to maximise profitability.

South African eggs are classified according to size, ranging from super jumbo to small eggs. The classification according to size can be seen in Table 2.1. The South African law also demands that eggs to be sold are graded according to a scale of grade one to three, with the highest grade being a grade one. A grade one egg should have no deformities and comply with all the characteristics of a highly graded egg. The full description of each egg grade can be found in the report on the regulations with regard to grading, packing and marketing of eggs destined for sale in the Republic of South Africa by the Minister of Agriculture, Forestry and Fisheries, under section 15 of the Agricultural Product Standards Act, 1990 (Act No. 119 of 1990).

Table 2.1 Egg classification according to size as specified by the Department of Agriculture, Forestry and Fisheries of South Africa (DAFF, 2014)

Size	Mass per egg in gram (g)
Super jumbo	More than 72 g
Jumbo	More than 66 g
Extra large	More than 59 g
Large	More than 51 g
Medium	More than 43 g
Small	More than 33 g

A variety of methods are used to measure egg quality. Indirect means of measuring egg quality makes use of methods such as specific gravity, non-destructive deformation, shell thickness and shell weight. In the commercial industry, the identification of cracks and other defects are done through candling, with the use of light or an electronic crack detector. Shell strength is measured through the composition of the shell, thickness of the shell and the weight of the shell (Roberts, 2004).

Albumen quality on the other hand is measured by the height of the thick albumen of the egg. This can be affected by the age of the bird, as well as the time the egg is in storage and storage conditions, with older eggs having a lower albumen quality (Silversides & Scott, 2001). The yolk quality is measured through its colour and the strength of the perivitelline membrane surrounding the yolk. A strong membrane will keep the yolk intact, resulting in a greater yolk height. The strength of the membrane decreases with age, making the yolk more susceptible to breaking (Jones & Musgrove, 2005).

The egg shell does not only hold the content of the egg, it is also the first defence against bacterial contamination and should therefore be in good condition in order to keep the contents safe for human consumption (Mabe *et al.*, 2003). Poor egg shell quality also increases the risk of cracked eggs during transport (Jones & Musgrove, 2005). Structurally damaged eggs cannot be sold commercially, therefore egg shell quality largely affects profitability.

Internal egg quality is an important factor of an egg for both producers and consumers. The time and temperature of storage, the strain and age of the hen, diet or nutrition, diseases, supplementation, ammonia exposure, induced moulting and medications are all factors that affect internal egg quality either independently or by interaction with each other (Roberts, 2004).

2.3 Nutrition

A balanced and complete layer hen diet consists of energy, protein, minerals, vitamins and additives. Feed additives form the smallest part of a complete layer diet; however they are highly effective in assisting production.

In order to achieve the required production and egg quality, various additives have been added to animal feed to combat natural and environmental stressors. Feed additives are ingredients that are commonly added to poultry feed, which may cause various responses, such as growth stimulation and improvement in the metabolism (Hashemi & Davoodi, 2011). They are said to enhance digestibility, nutrient absorption and eliminate pathogens in the gut, which in turn enhances productivity (Athanasiadou *et al.*, 2007). Antibiotics, immuno-stimulators, antioxidants, pH control agents and enzymes (Hashemi & Davoodi, 2011) are some of the most commonly used feed additives in animal nutrition. Others include trans-elements, vitamins, preservatives, colouring agents, flavouring agents, buffers, coccidiostats and natural plant extract (Hashemi & Davoodi, 2011).

The layer hen industry has relied heavily on antibiotics and probiotics to enhance egg production and egg quality parameters to meet industrial demands (Bedford, 2000). The most well-known feed additive, antibiotics, are commonly added to poultry feed to improve weight gain and the feed conversion ratio (FCR), and reduce disease susceptibility. This is done through improved gut health and absorption in the intestines. Antibiotics interaction with gut microbiota decreases the competition for nutrients and enhances nutrient digestibility due to a reduction in gut wall and *villus lamina propria*. They also reduce the microbial metabolites, which depresses growth and decreases the instances of pathogens and subclinical infections (Dibner and Richards., 2005). These responses give rise to a feed efficient and healthier bird, which in turn will improve egg production in layer hens.

The use of antibiotics, however, has numerous side effects, with the major concern being the development of drug-resistant bacteria that may transfer their resistance to pathogenic bacteria in animals and humans (Thacker, 2013). The presence of antibiotic residues in meat and milk products has also added to the view that antibiotic growth promoters are undesirable for animal product production (Cardozo *et al.*, 2004). The use of antibiotic growth promoters was banned in Europe in the year 2006 and the search for alternative sources of feed additives has commenced, which will assist in animal performance and production (Vondruskova *et al.*, 2010).

Another well-known feed additive used to improve production performance in layer hens is probiotics. The addition of probiotics in a layer diet has numerous responses. Probiotics helps to

maintain intestinal microflora by antagonistic activity and competitive exclusion of pathogenic bacteria in the intestine. Antagonistic activity eliminates pathogens by producing bactericidal substances (e.g., bacteriocins, organic acid and hydrogen peroxide), which supress growth of, and kills pathogens. Competitive exclusion on the other hand decreases the risk of salmonella infection. Probiotics also increase digestive enzymes activity and decrease bacterial enzyme activity and ammonia production. In addition, probiotics also promote feed intake and digestion, while neutralising enterotoxins and stimulating the immune system. These responses have proven to improved egg mass, egg weight and egg size (Jin *et al.*,1997), and numerous studies have found the supplementation of probiotics to increase the production of eggs and the feed conversion ratio of layers (Nahashon *et al.*, 1994a; 1994b; 1996a; 1996b; Mohan *et al.*, 1995; Tortuero and Fernandez, 1995; Abdulrahim *et al.*, 1996).

The effect of feed additives on egg quality is particularly important as egg quality determines the eggs' shelf life. The quality of eggs is also used to grade eggs and therefore has an influence on the selling potential of the eggs. As a result, natural feed additives have gained interest in recent years due to consumers' negative perception of synthetic feed additives and due to the use of antimicrobial additives being gradually phased out because of the negative side effects they have on animal and human health (Tipu *et al.*, 2006; Hashemi & Davoodi, 2011). This has led to the investigation of natural additives as products that may enhance production similar to synthetic additives. Therefore, testing the effect of natural feed additives in layer nutrition may prove to be effective in improving egg production and egg quality.

Limited experimental evidence however exists to accurately explain the effect of natural plants as feed additives. *In vitro* trials have reported anti-oxidative and antimicrobial responses, as well as immune stimulation (Hashemi & Davoodi, 2011). Further research on the use of plants as feed additives is therefore necessary. Fenugreek seed extract is one such natural feed additive that has shown potential to improve layer hen production due to its properties.

2.3.2 Fenugreek properties

Fenugreek (*Trigonella foenum-graecum* L) is a leguminous plant that grows annually in many countries (Hossain *et al.*, 2015), but was originally grown in the Middle East, North America and India (Park & Kim, 2015). Its properties have been used in human food and as a natural medical remedy for centuries (Hossain *et al.*, 2015). The seeds from the plant is small and hard, with a golden yellow colour (Jani *et al.*, 2009), and it has a distinct sweet (maple) and spicy flavour (Meghwal & Goswami, 2012). It is commonly used as a spice or pickling agent (Jani *et al.*, 2009).

Fenugreek is composed of carbohydrates (45-65%), dietary protein (20-30%), soluble fibres (15%) and fatty acids (5-10%) (Abbas, 2010). The plant also contains natural sources of vitamins and minerals (Abbas, 2010), such as calcium (Moradi kor *et al.*, 2013), alkaloids, flavonoids, saponins, as well as small amounts of volatile and fixed oils (Meghwal & Goswami, 2012). The presence of alkaloids gives the seeds a bitter taste (Fæste *et al.*, 2009), which can however be reduced through roasting (Meghwal & Goswami, 2012).

The seeds are high in soluble dietary fibre, which increases glucose metabolism in the digestive tract by lowering the rate of glucose absorption in the intestine and thus regulating blood sugar levels (Sharma *et al.*, 1990; Raju *et al.*, 2001). The endosperm of the seeds is rich in globulin, histidine, albumen and lecithin proteins (Mathur & Choudhry, 2009). A 100 g of endosperm has been found to contain 43.8 g of protein (Madhava Naidu *et al.*, 2011), and this protein is rich in lysine (Mathur & Choudhry, 2009).

However, fenugreek seed powder is a possible allergen and could be reactive with peanut allergens (Meghwal & Goswami, 2012). Jani *et al.* (2009) reported that although fenugreek seeds are non-toxic, it should not be consumed in excess or in levels above the prescribed recommendations. In addition, Garti *et al.* (1997) and Udayasekhara Rao *et al.* (1996) both reported that the drying of the fenugreek plant at high temperatures will reduce the chlorophyll, ascorbic acid and beta-carotene content of the seeds and leaves. These defects, together with the gummy and sticky texture of fenugreek seeds, cause problems during grinding (sticking to grinding wheel) and pelleting, which may result in choking (Sharma *et al.*, 1990).

2.3.4 Fenugreek as a natural feed additive

Fenugreek properties have been used in human food (to stimulate appetite and weight gain) and as a natural medical remedy for centuries (Hossain *et al.*, 2015). Humans experienced signs of increased hunger within 24 hours of receiving a dose of fenugreek leaf extract (Abdel-Barry *et al.*, 1997). The inclusion of fenugreek seed into the diet of rats has also motivated rats to eat and significantly increase feed intake due to the presence of an isolated steroidal saponin fraction (Petit *et al.*, 1993).

Fenugreek seeds consist of 4.8% saponins (Shah & Mir, 2004). Dioscin (saponin) is structurally similar to oestrogen and stimulates the pituitary gland to increase the secretion of growth hormones (GHs), as observed in a study with rats (Petit *et al.*, 1993). This is due to the ability of dioscin to bind to receptors on the pituitary gland, which activates GH secretion. The increased levels of GH in circulation positively affects muscle mass and strength, milk production and fat free body mass gain (Lee *et al.*, 2007).

Fenugreek's ability to increase feed intake may be attributed to its ability to increase sensitivity to insulin, which increases the serum concentration of ghrelin, the hormone that stimulates feed intake (Gad *et al.*, 2006). The degradation of ghrelin may, however, lead to a reduction in feed intake (Asakawa, 2005). Fenugreek also lowers the serum levels of low density lipoproteins (LDLs) (Sowmya & Rajyalakshmi, 1999). This is particularly important for the maintenance of ghrelin concentration, as LDLs interfere with the responses of ghrelin and lead to a decreased feed intake (De Vriese *et al.*, 2007). These possible mechanisms expressed by fenugreek plant extracts have encouraged its use as a natural feed additive. Fenugreek's effect in a layer hen diet is yet to be thoroughly explored. Therefore an investigation of fenugreek's possible effect as a natural feed additive on egg production and quality is necessary.

2.3.5 Nutrifen® and Nutrifen Plus®

Nutrifen® and Nutrifen Plus® are products produced from the seeds (cotyledon fraction) of the fenugreek plant. The cost of Nutrifen® in South Africa is ZAR 24/kg. Nutrifen Plus® costs significantly more than Nutrifen® at ZAR 40/kg, due to its added ingredients. The ingredients for the two products are as follows:

Nutrifen® composition:

- Fenugreek cotyledon concentrate (*Trigonella foenum Graecum*)

Nutrifen Plus® composition:

- 73% Fenugreek cotyledon concentrate (*T. foenum Graecum*)
- Fennel seed (*Foeniculum vulgane*)
- Saw Palmetto berries (*Serenoa repens*)
- Brown Kelp (*Laminariales*)
- MSM (natural source of Methylsulfonylmethane)
- White distilled vinegar powder

The use of fenugreek as a natural feed additive has been discussed in detail. Nutrifen Plus®, however, contains additional ingredients which may exert their unique effect on the egg production and quality. Fennel seed contain a sweeting component of 64.01%, 47.20% estragole and 16.81% trans-anethol pulse. This aromatic plant consists of a high percentage of fatty acids (Mohammed & Abbas, 2009). El-Deek *et al.*, (2003) reported an increased positive effect of fennel on body weight gain and FCR in broilers. Mohammed & Abbas (2009) also reported a significant improvement of a fenugreek-based diet on weight gain in broilers but found no significant effect of fennel seed on FCR.

Saw Palmetto berries (SPBEs) consist mainly of fatty acids and some phytosterols. The berries have a possible hepatotoxicity effect due to their antiandrogenic and estrogenic properties (Singh *et al.*, 2007). Saw Palmetto berries has been used traditionally to treat prostate problems and has also been tested for use on patients with benign prostatic hyperplasia. SPBEs have been proven to lower testosterone concentration and prostate-specific antigens, while the possibility of anti-inflammatory and anti-estrogenic effects may also exist (Goldmann *et al.*, 2001). Bennett & Hicklin (1998) observed in reports from earlier scientists that animals eating SPBE fruit in the wild had an increase in fat, and dairy cows consuming this fruit had richer milk. The fruits of SPBEs are rich in oils (rich in fatty acid content), of which oil cake is often made for consumption by livestock in the absence of good forage to increase

fattening (Bennett & Hicklin, 1998). These properties may affect egg production and egg quality positively when added to a layer hen diet.

Brown kelp forms part of the brown algae group, which are large in size and can be found in shallow waters, making it easy to harvest (Makkar *et al.*, 2015). In animal feed, kelp is a valuable supplement for nutricines, micro-minerals, carbohydrates (with prebiotic activity), pigments and polyunsaturated fatty acids (Evans & Critchley, 2014). Seaweed has been used medically as an iodine substitute and for intestinal disorders (El Gamal, 2010).

Brown algae (kelp) has shown potential in the wool production of sheep due to its sulphur-containing amino acids, methionine and cysteine (Mišurcová, 2012). In pig diets, brown algae has been found to improve pig health and meat quality. Brown algae has an organic source of iodine, which is easily metabolised and stored in pigs' muscles (Banoch, *et al.*, 2010). It enhances immune function, has a prebiotic effect that promotes gut health in pigs and has been found to cause an overall improvement in performance in piglets (O'Doherty *et al.*, 2010). Brown algae is also used in poultry feed to improve immune status, decrease microbial load in the digestive tract and increase quality of meat and eggs (Abudabos *et al.*, 2013). It has been shown to improve growth performance (Evans & Critchley, 2014) in broilers, while it was reported to cause decreased yolk cholesterol and increase carotene content in layers. At an inclusion level between 1 and 3%, green seaweed has shown to improved egg production and quality through increased egg weight, shell thickness and yolk colour (El-Deek & Al-Harthi, 2009). Bird health was also improved with the feeding of green seaweed due to lower *Escherichia coli* counts in faeces and an improved FCR (Wang, *et al.*, 2013). On the contrary, brown seaweed has shown no significant effect on body weight, egg weight, egg production, FCR and egg quality (El-Deek & Al-Harthi, 2009).

Methylsulfonylmethane (MSM) is composed of organic dietary sulphur and has been proven to have an anti-inflammatory and antioxidant effect when used in animal feed (Hwang *et al.*, 2017). Positive effects of MSM on the physiological indexes of animals have also been reported (Jiao *et al.*, 2017). In the diet of ducks, MSM has shown no significant effect on feed intake, body weight gain and FCR; however, a decrease in the mortality rate of ducks was found (Kim *et al.*, 2002; Hwang *et al.*, 2017). In contradiction, Hui-fang and Zhou (2008) found an increase in average daily gain and feed efficiency in ducks fed with MSM. Cho *et al.* (2006) reported improved feed efficiency and nutrient digestibility in pigs, while Jang *et al.* (2006) found an increase in average daily feed intake in pigs fed with 0.06% MSM. Lee *et al.* (2009) observed an increase in Fe, Cu and Zn in pigs supplemented with a MSM diet compared to those on the control diet.

The supplementation of MSM in broiler diets has been found to increase body weight and feed efficiency (Jiao *et al.*, 2017). Park *et al.* (2010) found that at an inclusion level of 0.1%, MSM significantly improved shell thickness and firmness, which in turn lowered the broken egg rate. The Haugh Unit (HU) and viscosity of the yolk and albumin, as well as egg freshness, was found to be increased with an increase in storage time at 4^0C. The overall egg texture and freshness/smell was also improved in eggs from MSM diets. However, when MSM is fed together with *Opuntia humifusa* (prickly pear), the positive effects on layer hen production and egg quality were eliminated (Park *et al.*, 2010). The positive traits of MSM in different animal diets indicates a possible positive effect of MSM in layer production and egg quality traits.

Distilled vinegar powder is a form of acetic acid. Organic acids are used in pig diets to improve growth rate and feed utilisation (Kirchgessner & Roth, 1982). Furuse & Okumura (1988) found that increasing acetic acid up to 2.45 g/kg in the form of powdered distilled vinegar and fed *ad lib* will significantly increase body weight gain of chicks. At acetic acid levels higher than 2.45 g/kg, the effects on production was detrimental (Furuse & Okumura, 1988).

2.3.6 Fenugreek in animal feed studies

Studies have been conducted to investigate the effect of fenugreek as an animal feed additive; these studies have reported the possibilities of improved animal performance, enhanced immune status and improved nutrient digestibility properties (Hossain *et al.*, 2015). There have been reports of fenugreek bringing about an increase in milk production in dairy cattle (Shah & Mir, 2004) and goats (Smit, 2014). Fenugreek has also been reported to increase live weight gain of growing pigs (Draghia-Akli & Fiorotto, 2004), and improve early weight gain and enhance dry matter and nitrogen retention of broiler chickens (Park & Kim, 2015).

In broiler breeders, an increase in semen quality and reproductive performance was observed with the addition of fenugreek in the diet (Abdel-Rahman *et al.*, 2014). This was also observed in layer hens, with reports of reproductive improvement (Alobaidy, 2012), and improved egg mass, egg quality (Hassan & Ragab, 2001), shell thickness and albumen percentage (Abaza, 2007). Furthermore, earlier sexual maturity in layers has also been reported (Awadein *et al.*, 2010).

In addition, fenugreek may have an effect on intestinal histomorphology, due to the antimicrobial action of the seeds (Qureshi *et al.*, 2015). This can reduce the inflammatory reaction at the mucosa, which assists villus growth (Mahmood *et al.*, 2015). Awadein *et al.* (2010) reported a reduction in the

total lipid content of the liver in layers on a diet substituted with 0.5% fenugreek. Fenugreek's hepato-protective and antioxidant activity enhances hepatic functioning (Bukhari *et al.*, 2008). Abdel-Rahman *et al.* (2014) observed an increase in the crypt depth, villus height and width, and surface area of the intestine of broilers that were fed a diet containing 5.33 kg/t of fenugreek. This will improve absorption due to the larger surface area, advance the utilisation of nutrients (Adil *et al.*, 2015) and improve overall gut health (Petrolli *et al.*, 2012).

An experiment conducted at the Dankook University in South Korea on the effect of Nutrifen® on egg production and egg quality, found that there was no significant difference in egg production between the control treatment and the Nutrifen® enriched diets (Dankook University, 2013). According to their findings, Nutrifen® therefore does not seem to affect the quantity of egg production. The experiment did however give conclusive evidence of the positive effect of Nutrifen® on egg quality. At an inclusion level of 0.9% Nutrifen® in a layer diet, the researchers found that Nutrifen® fed layers displayed an increase in egg weight, eggshell thickness, as well as an increase in yolk height and colour.

Hassan *et al.* (2004) studied the addition of 1% and 2% germinated and non-germinated fenugreek seeds in the diet of layer hens and concluded that there was no significant effect of the germinated treatment on egg quality. However, egg production increased economically with the addition of both treatment variations. Abaza (2007) also observed a 2.23% increase in egg production compared to the control. On the other hand, Criste *et al.* (2013) reported that with inclusion levels of 1% and 2% fenugreek in the layer diet, the 2% concentration showed significantly lower egg production. The egg production achieved by the 1% and 2% concentrations were also observed to be significantly lower than the egg production achieved by the control group. In addition, lower concentration of serum cholesterol and triglycerides were observed in the 2% concentration treatment group (Criste *et al.*, 2013).

Panaite *et al.* (2015) fed 2% and 1% fenugreek diets to layers and found that the physical parameters in terms of egg weight did not differ significantly between treatments, which contradicts the research at Dankook University. The yolk weight increased for the 2% fenugreek concentration group, while for the control group, albumen weight was observed to be greater than the 1% concentration group (Panaite *et al.*, 2015).

In the diets of broilers, no significant changes were observed in the FCR of the birds fed 0.1% (Park & Kim, 2015) and 0.3% (Abbas, 2010) of fenugreek-supplemented diets respectively, compared to the control groups. The FCR in broilers is particularly important for improved production, therefore an improvement in the FCR will improve profitability. In addition, no improvement of feed intake was

observed (Abbas, 2010). The plasma cholesterol of birds fed the fenugreek treatment diets was found to be reduced (Abdel-Rasoul & Yousif, 2003).

2.4 Other factors affecting egg quality

Nutrition and feed additives do not act alone to determine egg production and egg quality. The egg production of layers is also affected by the physical condition of the hen in terms of her age, body condition and health. In addition to this, environmental stressors play a vital role in determining egg production and quality. The egg quality may also be further affected by the storage condition and length of storage after the eggs are laid. These above-mentioned factors can therefore alter the egg production and egg quality despite genetics and optimum nutrition. A positive effect or reaction of a natural feed additive such as fenugreek on these above-mentioned factors may also increase fenugreeks' ability to improve egg production and quality.

2.4.1 Hen age

The age of the hen can affect the egg shell and egg weight, as well as the albumen and yolk quantity and quality. Deformed eggs are common in older hens due to the weakening of muscular tone of the shell gland and changes in the ratio of the thick to thin albumen (Travel & Nys, 2011). Older birds lay larger eggs with thinner shells due to difficulty extracting calcium from their bones (Butcher & Miles, 2015). In addition, the weight of an egg also increases with an increase in the age of a bird (Travel & Nys, 2011).

Albumen quality is measured by the height of the thick albumen of the egg. In older hens, the albumen quality is much lower (Silversides & Scott, 2001), and heavier albumen is observed in their eggs due to a decrease in albumen solids, causing the weight of the albumen to increase (Travel & Nys, 2011). On the other hand, the yolk quality is measured through its colour and the strength of the perivitelline membrane surrounding the yolk. A strong membrane will keep the yolk intact, resulting in a greater yolk height. The strength of the membrane decreases with age, making the yolk more susceptible to breaking (Jones & Musgrove, 2005).

However, young birds are prone to produce small or shell-less eggs early in their production cycle; this may be due to younger birds using dietary calcium for skeletal development rather than for egg

production (Butcher & Miles, 2015). In addition, double-yoked eggs, which are caused by multiple ovulations, is a very common occurrence in young birds (Travel & Nys, 2011).

Fenugreek is a natural source of calcium. The addition of fenugreek may therefore positively improve egg production in old and young birds.

2.4.2 Body condition

The body weight or condition of a hen is a characteristic that has economic importance in the layer industry. Although layer hen growth is not as important as in broilers, it remains an important part of the hen's production cycle (Di Masso *et al.*, 1998). The frame size of the hen can influence its egg production. Small hens tend to have difficulty laying and this can result in defects, such as small and deformed eggs, dystocia and death. On the other hand, large birds have increased maintenance requirements and therefore also an increased cost of production (Di Masso *et al.*, 1998).

The weight of the hen at the onset of production determines future production. A low asymptotic weight and a high maturing weight is an advantage for farmers. Hens that mature early and are light in weight would have a shorter pre-sexual, non-reproductive phase, and the cost of maintenance would decrease. This may also assist the rapid onset of lay, regular laying patterns and a uniform egg weight range (Di Masso *et al.*, 1998). The onset of sexual maturity is around 15 weeks in the Hy-Line hens and the optimum body weight at this age was determined to be between 1.261 kg and 1.339 kg. When the correct body weight (1.40-1.48 kg) is achieved and the uniformity of a pullet flock entering egg production is higher than 90%, the flock will perform optimally during the production period (Hy-Line International, 2016). The characteristics for body weight at sexual maturity does however differ between breeds (Du Plessis & Erasmus, 1972).

Feugreek's reported response of stimulating feed intake and also increasing body weight may positively contribute to improving production of thin birds.

2.4.3 Health

Most diseases negatively affect egg production, with the most severe diseases being pathogens that grow in the reproductive tract of the hen. Egg discolouration and deformities (soft shell, watery egg whites and rough shell) may result from infectious bronchitis and egg drop syndrome (Jacob *et al.*, 2003; Hy-Line International, 2016). Other diseases that negatively affect egg production and quality include

Newcastle disease (Augusto Do Amaral, 2004), cage layer fatigue, fatty liver syndrome, rickets and avian influenza (Jacob *et al.*, 2003).

The health status of the intestinal tract of a layer is particularly important for maintenance, growth and reproduction, because a healthy intestinal tract means more efficient absorption of feed in the tract. There are numerous studies that evaluate fenugreek's effect on the health status of the gut. This response can positively improve production and egg quality if gut health is improved from the addition of fenugreek to the diet.

2.4.4 Stress

The bacterial population in the tract needs to be well balanced, however, during stress this balance is disturbed. Stress on a layer hen can be in the form of compromised health (as discussed above) environmental stress (e.g., temperature and humidity), a change in feed and transportation. Stress has a critical negative effect on egg production and egg quality.

Environmental stressors such as overcrowding and overheating cause an imbalance in the intestinal microflora of the bird and decrease the immune function (Jin *et al.*, 1997). Stress resulting from high population densities causes an increase in body-checked eggs, which is as a result of the contraction of shell glands during the early development of the egg shell (Reynard & Savory, 1999).

On the other hand, the climate plays an important role in egg production, with birds in humid and warm climates producing an average of 180 to 200 eggs per year, while hens in more temperate climates can achieve significantly higher production of between 250 to 300 eggs per year (Zaheer, 2015).

Heat stress may be caused by factors such as high air temperature and humidity, which is detrimental for layer production (Lara & Rostagno, 2013). Heat stress reduces feed intake and therefore negatively affects growth, egg production and egg quality. Environmental stress and heat stress results in eggs being retained beyond the normal time for oviposition, thus causing a white chalky layer on the egg shell (Hughes *et al.*, 1986) and a reduction in shell pigment (Lang & Wells, 1987). Combating periods of heat stress is therefore important to maintain high egg quality and production.

The impact of heat stress can be reduced through good management practices such as keeping the house cool and supplying cool water (Roberts, 2004). Dietary energy, protein and amino acid manipulation will also reduce the side effects of thermal stress (Travel & Nys, 2011). Similarly, the supply of vitamin C and probiotics have proven to reduce the risk of heat stress (Zhang *et al.*, 2017). Marsden

& Morris (1987) suggest that 22-24^{0}C is the optimal temperature range for egg production. At temperatures higher than 29^{0}C, the intake, metabolism, production and egg quality of the bird will decrease (Marsden & Morris, 1987). At temperatures lower than 10^{0}C, the birds will over eat (Travel & Nys, 2011).

In addition, the stress of excessive handling and the relocation of the birds also adds a stress factor, which may lead to an increase in the number of cracked eggs, and a higher value of calcium dusted eggs, white banded eggs, slab-sided eggs and misshapen eggs (Reynard & Savory, 1999).

Hen health is of utmost importance for the maintenance of long-term egg production. Hen health during production has been maintained with the use of antioxidants to reduce the effect of stress by strengthening immune responses against heat stress (Asli *et al.*, 2007). Commonly used antioxidants in layer diets have been vitamin E and C (Puthpongsiriporn *et al.*, 2001) and Zn, Cu, Mn and Se (Bülbül *et al.*, 2008). Alternative sources of antioxidants are being investigated, including those from plant sources that have shown significant potential. The safety and efficiency of these sources are being considered as a viable antioxidant source. Fenugreek has been reported to express similar effects as those expressed by antioxidants (Bukhari *et al.*, 2008). This may therefore lead to improved stress handling of the bird, which in turn means that stress has a smaller effect on egg production and egg quality.

2.4.5 Egg storage

During storage, eggs lose carbon dioxide (CO_2) through the shell over time. This causes the egg albumen content to become transparent and watery. The effect of CO_2 losses through the shell is increased with higher temperatures (Benton & Brake, 2000). The longer the egg spends in storage, the larger the air sac will be, and therefore more moisture and CO_2 will escape and be lost from the egg (Travel & Nys, 2011).

The degradation of albumen quality may be related to an increase in acidity (i.e., decrease in pH) in the thick albumen (Scott & Silversides, 2000). Albumen quality has been shown to reduce when eggs are stored at 50-60% humidity and at temperatures of 7-13^{0}C (Gerber, 2009). The quality of the HU also decreases by 10-15 HU during the first few days of storage and 30 HU by the end of 30 days, when eggs are stored at humidity levels lower than 70% (Okeudo *et al.*, 2003).

The shape of the yolk should be round and firm under ideal conditions (Jones, 2006); however, storage has been proven to negatively affect egg yolk quality by decreasing the yolk membrane's strength (Jones *et al.*, 2002). During storage, if the internal temperature of the egg is higher than 7^{0}C,

the vitelline membrane enclosing the yolk, the protein structure of the yolk and protein structure of the thick albumen degrades much faster. This allows water and albumen protein to enter the yolk, causing severe mottling (Kirunda & McKee, 2000). Jones and Musgrove (2005) further found that the yolk membrane's elasticity decreased during storage, which may lead to a high instance of broken yolks (Jones & Musgrove, 2005). Extended cooling or freezing of eggs may also result in rubbery yolk (Ahmadi & Rahimi, 2011).

In addition, the physical properties and taste of the eggs are also altered during storage. Samli *et al.* (2005) reported a decrease in the viscosity, taste and flavour of older eggs. Storage is an important practice and eggs should therefore be stored appropriately in order to preserve them. In contrast to this, the egg shell strength seemed to be unaffected by the extended storage time at cold temperatures (Jones & Musgrove, 2005).

Various methods for maintaining egg quality during storage have been developed. Jones & Musgrove (2005) found that when eggs are stored at 4^0C and 80% relative humidity, the albumen quality and vitelline membrane strength may be preserved for up to 10 weeks (Jones & Musgrove, 2005). On the other hand, Keener *et al.* (2000) found that rapid cooling using CO_2 and storage in CO_2-modified atmospheres may also increase shelf life to more than 14 weeks.

2.5 Conclusion

In the year 2050, the world will require 60-70% more animal products than currently required and produced (Makkar *et al.*, 2015). This will lead to an increase in demand for table eggs. This, together with increased pressure for sustainable and safe food production from consumers, will make this task increasingly difficult.

Optimum egg production and the maintenance of egg quality is therefore particularly important to meet the future demands required. Production, however, can be easily compromised with inadequate nutrition, hen age, body condition, environmental stress, hen health and the storage time and condition of the eggs. These factors need to be addressed, and therefore feed additives have been added to layer diets to combat the stressors that hens face during production. Due to the negative light shed on synthetic feed additives such as antibiotics, researching natural alternatives to improve the production and egg quality of layer hens has become important. Natural feed additives such a fenugreek have the potential to reduce stress factors and boost production through improved growth and feed intake, as well as improved egg quality. Unfortunately, limited evidence exists to explain fenugreek's possible effect on maintaining egg quality during storage. Therefore, the purpose of this research is to investigate the effect of fenugreek products Nutrifen® and Nutrifen Plus® on layer hen production and egg quality parameters during the storage thereof.

2.6 References

Abaza, I.M., 2007. Effects of using fenugreek, chamomile and radish as feed additives on productive performance and digestibility coefficients of laying hens. Egypt. Poult. Sci. 27, 199-218.

Abbas, R.J., 2010. Effect of using fenugreek, parsley and sweet basil seeds as feed additives on the performance of broiler chickens. Int. J. Poult. Sci. 9(3), 278-282.

Abdel-Barry, J.A., Abdel-Hassan, I.A. & Al-Hakiem, M.H.H., 1997. Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. J. Ethnopharmacol. 58(3), 149-155.

Abdel-Rahman, H.A., Fathallah, S.I., Helal, M.A., Nafeaa, A.A. & Zahran, I.S., 2014. Effect of turmeric (*curcuma Longa*), fenugreek (*trigonella foenum-graecum L*.) and/or bioflavonoid supplementation to the broiler chicks' diet and drinking water on the growth performance and intestinal morphometeric parameters. Glob. Vet. 12(5), 627-635.

Abdel-Rasoul, E. & Yousif, W., 2003. Effect of fenugreek (*Trigonella foenum-graecum*) seed powder (as capsules) on certain physiological aspects of broiler chickens treated with vanadyl sulphate. Iraqi. J. Vet. Sci. 17, 101-109.

Abdulrahim S.M., Haddadin M.S.Y., Hashlamoun E.A.R., Robinson R.K., 1996. The influence of *Lactobacillusacidophilus* and *bacitracin* on layer performance of chickens and cholesterol content of plasma and egg yolk. British Poultry Science: 341-346.

Abudabos, A.M., Okab, A.B., Aljumaah, R.S., Samara, E.M., Abdoun, K.A. & Al-Haidary, A.A., 2013. Nutritional value of green seaweed (*Ulva lactuca*) for broiler chickens. Ital. J. Anim. Sci. 12(2), 177-181.

Adil, S., Qureshi, S. & Pattoo, R., 2015. Review on positive effects of fenugreek as poultry feed additive. Int. J. Poult. Sci. 14(12), 664-669.

Ahmadi, F. & Rahimi, F., 2011. Factors affecting quality and quantity of egg production in laying hens: A Review. World Appl. Sci. J. 12(3), 372-384.

Ajakaiye, J., Pérez, A. & Mollineda, A., 2011. Effects of high temperature on production in layer chickens supplemented with vitamins C and E. Efectos de la alta temperatura sobre la producción en gallinas. Rev.MVZ Córdoba. 16(1), 2283-2291.

Alagawany, M., El-Hack, M.E.A., Farag, M.R., Tiwari, R., Sachan, S., Karthik, K. & Dhama, K., 2016. Positive and negative impacts of dietary protein levels in laying hens. Asian J. Anim. Sci. 10(2), 165-174.

Almeida, V., Dias, A., Bueno, C., Couto, F., Rodrigues, P., Nogueira, W. & Faria Filho, D., 2012. Crude protein and metabolizable energy levels for layers reared in hot climates. Rev. Bras. Ciência Avícola. 14(3), 203-208.

Alobaidy, R., 2012. Effect of fenugreek seeds and olive leaves ration supplementation on productive and physiological performance of laying breeder hen. MSc thesis submitted to the College of Agriculture and Forestry, University of Mosul, Iraq. pp. 1-40.

Asakawa, A., 2005. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. Gut. 54(1), 18-24.

Asli, M.M., Hosseini, S.A., Lotfollahian, H. & Shariatmadari, F., 2007. Effect of probiotics, yeast, vitamin E and vitamin C supplements on performance and immune response of laying hen during high environmental temperature. Int. J. Poult. Sci. 6(12), 895-900.

Athanasiadou, S., Githiori, J. & Kyriazakis, I., 2007. Medicinal plants for helminth parasite control: Facts and fiction. Anim. Consort. 1(9), 1392-1400.

Augusto Do Amaral, L., 2004. Drinking water as a risk factor to poultry health. Brazilian J. Poult. Sci. Rev. Bras. 6(4), 191-199.

Awadein, N.B., Eid, Y.Z. & AbdEl-ghany, F.A., 2010. Effect of dietary supplementation with phytoestrogens sources before sexual maturity on productive performance of mandarah hens. Egypt. Poult. Sci. 30(3), 829-846.

Banoch, T., Fajt, Z., Drabek, J. & Svoboda, M., 2010. Iodine and its importance in human and pigs. Veterinarstvi. 60, 690-694.

Bedford, M., 2000. Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimize subsequent problems. World's Poult. Sci. J. 56:347-365.

Bennett, B.C. & Hicklin, J.R., 1998. Uses of saw palmetto (*Serenoa repens, Arecaceae*) in Florida. Econ. Bot. 52(4), 381-393.

Benton, C.E. & Brake, J., 2000. Effects of atmospheric ammonia on albumen height and pH of fresh broiler breeder eggs 1. Poult. Sci. 79, 1562-1565.

Bregendahl, K., Roberts, S.A., Kerr, B. & Hoehler, D., 2008. Tryptophan and valine relative to lysine for White Leghorn-Type laying hens of twenty-eight to thirty-four weeks of age. Poult. Sci. 87, 744-758.

Browning, L. & Cowieson, A., 2015. Interactive effects of vitamin D3 and strontium on performance, nutrient retention, and bone mineral composition in laying hens. J. Sci. Food. Agric. 95, 1080-1087.

Bukhari, S.B., Bhanger, M.I. & Memon, S., 2008. Antioxidative activity of extracts from fenugreek seeds (*Trigonella foenum-graecum*). J. Anal. Environ. Chem. 9(2), 78-83.

Bülbül, A., Bülbül, T., Kucukersan, S., Sireli, M. & Eryavuz, A., 2008. Effects of dietary supplementation of organic and inorganic Zn, Cu and Mn on oxidant/antioxidant balance in laying hens. Kafkas Univ Fak Derg. 14(1), 19-24.

Bunchasak, C., Poosuwan, K., Nukraew, R., Markvichitr, K. & Choothesa, A., 2005. Effect of dietary protein on egg production and immunity responses of laying hens during peak production period. Int. J. Poult. Sci. 4(9), 701-708.

Butcher, G.D. & Miles, R., 2015. Concepts of eggshell quality 1 concepts of eggshell quality. IFAS Ext. Univ. Florida. (June), 1-2.

Butler, E.J., Curtis, M.J., Pearson, A.W. & Mcdougall, J.S., 1972. Effect of infectious bronchitis on the structure and composition of egg albumen. J. Sci. Fd. Agric. 23, 359-369.

Calderon, V.M. & Jensen, L.E.O.S., 1990. The requirement for sulfur amino acid by laying hens as influenced by the protein concentration. Poult. Sci. 69, 934-944.

Calvery, H. O. & Titus, H. W., 1934. The composition of the proteins of eggs from hens on different diets. J. Bio. Chem. 105, 683-689.

Cardozo, P.W., Calsamiglia, S., Ferret, A. & Kamel, C., 2004. Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. J. Anim. Sci. 82(11), 3230-3236.

Carvalho Mello, H.H., Gomes, P.C., Rocha, T.C., Donzele, J.L., Almeida, R.L., Troni, A.R., Carvalho, B.R. & Silva Viana, G., 2012. Determination of digestible isoleucine: lysine ratio in diets for laying hens aged 42-58 weeks. Rev. Bras. Zootec. 41(5), 1313-1317.

Cho, J.H., Chen, Y.J., Min, B.J., Kim, H.J., Kwon, O.S., Shon, K.S., Kim, I.H., Kim, S.J. & Asamer, A., 2006. Effects of essential oils supplementation on growth performance, IgG concentration and fecal noxious gas concentration of weaned pigs. Asian-Australasian J. Anim. Sci. 19(1), 80-85.

Criste, R.D., Panaite, T., Bercaru, A., Varzaru, I., Ropota, M. & Cornescu, G.M., 2013. Study on the use of fenugreek in laying hens' diets on egg quality. 19th Eur. Symp. Poult. Nutr. Potsdam. Ger. 1-5.

Dankook University, 2013. Effects of Nutrifen on egg production, quality, nutrient digestibility, blood profiles, fecal microflora, and fecal noxious gas emission in laying hens. Department of Animal Resource and Science, Dankook University, Republic of Korea.

De Vriese, C., Hacquebard, M., Gregoire, F., Carpentier, Y. & Delporte, C., 2007. Ghrelin interacts with human plasma lipoproteins. Endocrinology. 148(5), 2355-2362.

Di Masso, R.J., Dottavio, M., Canet, Z.E. & Font, M.T., 1998. Body weight and egg weight dynamics in layers. Poult. Sci. 77(6), 791-796

Dibner, T.T., and Richards, J.D. (2005). Antimicrobial growth promoters in agriculture: History and mode of action. Poultry Science 84: 634-643.

Domingues, C.H.F., Sgavioli, S., Praes, M.F.F.M., Duarte, K.F., Castiblanco, D.M.C., Santos, E.T., Alva, J.C.R. & Junqueira, O.M., 2012. Lysine and methionine + cystine for laying hens during the post molting phase. Rev. Bras. Ciência Avícola. 14(3), 187-192.

Draghia-Akli, R. & Fiorotto, M., 2004. A new plasmid-mediated approach to supplement somatotropin production in pigs. J. Animal. Sci. 82(13), 264-269.

Du Plessis, P. & Erasmus, J., 1972. The relationship between egg production egg weight and body weight in laying hens. Worlds. Poult. Sci. J. 28(3), 301-310.

El Gamal, A., 2010. Biological importance of marine algae. In: S. Kim, ed. Handbook of marine algae biotechnology and applied phycology. Saudi Pharmaceutical Journal, 18(1), pp.1-25.

El-Deek, A. & Al-Harthi, M., 2009. Nutritive value of treated brown marine algae in pullet and laying diets. In: World Poultry Science Association, Proceedings of the 19th European Symposium on Quality of Poultry Meat, 13th European Symposium on Quality of Eggs and Egg Products, Turku, Finland, 21-25 June 2009. pp. 1-12.

EL-Deek, A., Attia,Y.A, A and Hannfy, M., 2003. Effect of anise (*Pimpinella anisiumj*), ginger (*Zingiber officinale roscoe*) and fennel (*Foeniculum vulgare*) and their mixture of performance of broilers. Arch. Geflugelk. 67, 92-96.

Evans, F.D. & Critchley, A.T., 2014. Seaweeds for animal production use. J. Appl. Phycol. 26(2), 891-899.

Fæste, C.K., Namork, E. & Lindvik, H., 2009. Allergenicity and antigenicity of fenugreek (*Trigonella foenum-graecum*) proteins in foods. J. Allergy Clin. Immunol. 123(1), 187-194.

Forbes, J. M., 2007. Voluntary food intake and diet selection in farm animals. 1st ed. CABI Oxfordshire, UK.

Furuse, M. & Okumura, J., 1988. Effect of dietary acetic acid levels on protein and energy utilization in chicks. Poult. Sci. 68, 795-798.

Gad, M.Z., El-Sawalhi, M.M., Ismail, M.F. & El-Tanbouly, N.D., 2006. Biochemical study of the anti-diabetic action of the egyptian plants fenugreek and balanites. Mol. Cell. Biochem. 281(1 2), 173-183.

Garti, N., Madar, Z., Aserin, A. & Sternheim, B., 1997. Fenugreek galactomannans as food emulsifiers. LWT - Food Sci. Tech. 30, 305-311.

Gerber, N., 2009. Factors affecting egg quality in the commercial laying hen: A review. Poult. Ind. Assoc. New Zeal. 1-28.

Goldmann, W.H., Sharma, A.L., Currier, S.J., Johnston, P.D., Rana, A. & Sharma, C.P., 2001. Saw Palmetto berry extract inhibits cell growth and cox-2 expression in prostatic cancer cells. Cell Biol. Int. 25(11), 1117-1124.

Hashemi, S.R. & Davoodi, H., 2011. Herbal plants and their derivatives as growth and health promoters in animal nutrition. Vet Res Commun. 35, 169-180.

Hassan, H.A. & Ragab, M.S., 2001. The productive performance and egg quality of laying hens under the effect of dietary fenugreek seeds (ungerminated and germinated) and different dietary protein levels. Egypt. Poult. Sci. 1-14.

Hassan, I., Askar, A. & El-Shourbagy, G., 2004. Influence of some medicinal plants on performance; physiological and meat quality traits of broiler chicks. Poultr. Sci. 24, 247-266.

Hossain, M.M., Begum, M., Nyachoti, C.M., Hancock, J.D. & Kim, I.H., 2015. Dietary fenugreek seed extract improves performance and reduces fecal *E. coli* counts and fecal gas emission in lactating sows and suckling piglets. Can. J. Anim. Sci. 95(4), 561-568.

Hughes, B.O., Gilbert, A.B. & Brown, M.F., 1986. Categorisation and causes of abnormal egg shells: Relationship with stress. Br. Poult. Sci. 27(2), 325-337.

Hui-fang, L. & Zhou, A., 2008. Effects of plant extracts, cysteamine and methylsulfonylmethane on productive performance and slaughter characteristics in meat ducks. Nat. Prod. Res. Dev., 20, 302-306.

Hwang, J., Cheong, S.H., Kim, Y., Lee, J., You, B., Moon, S., Jeon, B. & Park, P., 2017. Effects of dietary supplementation of oriental herbal medicine residue and methyl sulfonyl methane on the growth performance and meat quality of ducks. Anim. Prod. Sci. 57, 948-957.

Hy-Line International. 2016. Hy-Line Brown commercial layers management guide. Hy-Line Brown Commer. Layers Manag. Guid. 1-44. Available at: https://www.hyline.com/UserDocs/Pages/BRN_COM_ENG.pdf [Accessed 26.11.2018].

Jacob, J.P., Wilson, H.R., Miles, R.D., Butcher, G.D. & Mather, F.B., 2003. Factors affecting egg production in backyard chicken flocks. EDIS Publ. Syst. 1-8. Available at http://edis.ifas.ufl.edu/ps029 [Accessed 26.11.2018].

Jang, H.D., Oo, J.S., Chae, S.J., Park, S.L., Kim, I.C., Park, J.C., Jung, H.J., Kim, Y.H., Kim, I.H. & Seok., H.B., 2006. Effects of dietary of methyl sulfonyl methane on growth performance and meat quality characteristics in growing-finishing pigs. Korean J. Intl. Agri. 18, 116-120.

Jani, R., Udipi, S.A. & Ghugre, P.S., 2009. Mineral content of complementary foods. Indian J. Pediatr. 76(1), 37-44.

Jensen, V. M. & Calderon, L. S., 1990. The requirement for sulfur amino acid by laying hens as influenced by the protein concentration. Poultr. Sci. 69, 934-944.

Jiao, Y., Park, J.H., Kim, Y.M. & Kim, I.H., 2017. Effects of dietary methyl sulfonyl methane (MSM) supplementation on growth performance, nutrient digestibility, meat quality, excreta microbiota, excreta gas emission, and blood profiles in broilers. Poult. Sci. (0), 1-8.

Jin, L. Z., Ho, Y. W., Abdul lah, N., and Jalaludin, S., 1997. Probiotics in poultry: Modes of action. World's Poult. Sci. J., 53, 351-368.

Johnson, A.L., 2000. Reproduction in the female. In: Sturkie's Avian Physiology (5th ed.). Ed: Whittow, G.C., University of Hawaii at Manoa, Honolulu, Hawaii. pp. 569.

Jones, D.R. & Musgrove, M.T., 2005. Effects of extended storage on egg quality factors. Poult. Sci. 84, (2001), 1774-1777.

Jones, D.R., 2006. Conserving and monitoring shell egg quality. Aust. Poult. Sci. Symp. 18, 157-165.

Jones, D.R., Tharrington, J.B., Curtis, P.A., Anderson, K.E., Keener, K.M. & Jones, F.T., 2002. Effects of cryogenic cooling of shell eggs on egg quality. Poult. Sci. 81(5), 727-733.

Jones, J., 1990. Production and egg-quality responses of White Leghorn layers to anticoccidial agents. Poultr. Sci. 69(3), 378-387.

Junqueira, O.M., De Laurentiz, A.C., Da Silva Filardi, R., Rodrigues, E.A. & Casartelli, E.M., 2006. Effects of energy and protein levels on egg quality and performance of laying hens at early second production cycle. J. Appl. Poult. Res. 15(1), 110-115.

Keener, K.M., LaCrosse, J.D., Curtis, P., Anderson, K.E. & Farkas, B.E., 2000. The influence of rapid air cooling and carbon dioxide cooling and subsequent storage in air and carbon dioxide on shell egg quality. Poult. Sci. 79(7), 1067-1071.

Kim, B., Hwing, I., Kang, S., Shin, S., Woo, S. & Kim, Y., 2002. Effect of dietary ginseng, *Dioscorea japonica*, and oriental medicine refuse on productivity of Korean native chicken. Korean J. Anim. Sci. Technol. 44, 297-304.

Kirchgessner, M. & Roth, F., 1982. Fumaric acid as a feed additive in pig nutrition. Pig News Inform. 3, 259-264.

Kirunda, D. & McKee, S., 2000. Relating quality characteristics of aged eggs and fresh eggs to vitelline membrane strength as determined by a texture analyzer. Poultr. Sci. 79, 1189-1193.

Lang, M.R. & Wells, J.W., 1987. A review of eggshell pigmentation. World. Poult. Sci. J. 43(3), 238-246.

Lara, L.J. & Rostagno, M.H., 2013. Impact of heat stress on poultry production. Animals. 3(2), 356-369.

Lee, J.I., Min, H., Lee, J., Jeong, J., Ha, Y., Kwack, S. & Park, J., 2009. Changes in the quality of loin from pigs supplemented with dietary methyl sulfonyl methane during cold storage. Korean J. Food Sci. An. 29, 229-237.

Lee, L.T.O., Siu, F.K.Y., Tam, J.K.V, Lau, I.T.Y., Wong, A.O.L., Lin, M.C.M., Vaudry, H. & Chow, B.K.C., 2007. Discovery of growth hormone-releasing hormones and receptors in nonmammalian vertebrates. Proc. Natl. Acad. Sci. U.S.A. 104(7), 2133-2138.

Leeson, S., Summers, J. & Diaz, G., 2000. Nutrición Aviar Comercial. Santafé de Bogotá, Colombia, pp. 359.

Liu, X., Zhang, Y., Yan, P., Shi, T. & Wei, X., 2016. Effects of dietary conjugated linoleic acid on the performance of laying hens, fatty acid composition of egg yolk, egg flavor, and some serum hormones. Ajas. 11(3), 11.

Lunven, P., Marcq, C., Carnovale, E. & Fratoni, A., 1973. Amino acid composition of hen's egg. Br. F. Nutr. 30, 189.

Mabe, I., Rapp, C., Bain, M.M. & Nys, Y., 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poult. Sci. 82(12), 1903-1913.

Madhava Naidu, M., Shyamala, B.N., Pura Naik, J., Sulochanamma, G. & Srinivas, P., 2011. Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. LWT - Food Sci. Technol. 44(2), 451-456.

Mahmood, S., Rehman, A., Yousaf, M., Akhtar, P., Abbas, G., Hayat, K., Mahmood, A. & Shahzad, M.K., 2015. Comparative efficacy of different herbal plant's leaf extract on haematology, intestinal histomorphology and nutrient digestibility in broilers. Adv. Zool. Bot. 3(2), 11-16.

Makkar, H.P.S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F. & Ankers, P., 2015. Seaweeds for livestock diets: a review. Anim. Feed Sci. Technol. 1-24.

Marsden, A. & Morris, T.R., 1987. Quantitative review of the effects of environmental temperature on food intake, egg output and energy balance in laying pullets. Br. Poult. Sci. 28(4), 693-704.

Mathur, P. & Choudhry, M., 2009. Consumption pattern of fenugreek seeds in Rajasthani families. J. Hum. Ecol. 25(1), 9-12.

Mazzuco, H. & Bertechini, A.G., 2014. Critical points on egg production: causes, importance and incidence of eggshell breakage and defects. Sci. Agrotechnology. 38(1), 7-14.

Mcdonald, M.W., 1979. Lysine and methionine supplements in diets for laying pullets. Aust. J. Agric. Research. 30, 983-990.

Meghwal, M. & Goswami, T.K., 2012. A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. J. Food Process. Technol. 3(9), 1-10.

Mišurcová, L., 2012. Chemical composition of seaweeds. In: S. Kim, ed. Handbook of marine macroalgae: Biotechnology and applied phycology. John Wiley & Sons Ltd, UK. pp. 567.

Mohammed, A.A. & Abbas, R.J., 2009. The effect of using fennel seeds (*Foeniculum vulgare L.*) on productive performance of broiler chickens. Int. J. Poult. Sci. 8(7), 642-644.

Mohan, B., Kadirvel, R., Bhaskaran, M. & Natarajan, A., 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. Brit. Poult. Sci. 36, 799-803.

Moradi Kor, N., Didarshetaban, M.B. & Pour, H.R.S., 2013. Fenugreek (*Trigonella foenum-graecum L.*) as a valuable medicinal plant. Int. J. Adv. Biol. Biomed. Res. 1(8), 922931.

Nahashon, S.N., Nakaue, H.S & Mirosh, L.W. 1994a. Phytase activity, phosphorous and calcium retention, and performance of single comb White Leghorn layers fed diets containing two levels of available phosphorous and supplemented with direct-fed microbials. Poult, Sci, 73, 1552-1562.

Nahashon, S.N., Nakaue, H.S & Mirosh, L.W. 1994b. Production variables and nutrient retention in single comb White Leghorn laying pullets fed diets supplemented with direct-fed microbials. Poult. Sci. 73, 1699-1711.

Nahashon, S.N., Nakaue, H.S & Mirosh, L.W. 1996a. Nutrient retention and production parameters of single comb White Leghorn layers fed diets with varying crude protein levels and supplemented with direct-fed micrombials. Anim. Feed Sci. Technol. 61, 17-26.

Nahashon, S.N., Nakaue, H.S & Mirosh, L.W. 1996b. Performance of single comb White Leghorn layers fed a diet with a live microbial during the growth and egg laying phases. Anim. Feed Sci. Technol. 57, 25-38.

Novak, C., Yakout, H. & Scheideler, S., 2004. The combined effects of dietary lysine and total sulfur amino acid level on egg production parameters and egg components in Dekalb Delta laying hens. Poult. Sci. 83(6), 977-984.

O'Doherty, J.V., McDonnell, P. & Figat, S., 2010. The effect of dietary laminarin and fucoidan in the diet of the weanling piglet on performance and selected faecal microbial populations. Livest. Sci. 134, (13), 208-210.

Okeudo, N., Onwuchekwa, C. & Okoli, I., 2003. Effect of oil treatment and length of storage on the internal quality, organoleptic attributes and microbial profile of chicken eggs. Tropic. Animal Prod. Invest. 6, 63-70.

Panaite, T.D., Varzaru, I., Criste, R.D., Cornescu, G.M., Ropota, M. & Predescu, C., 2015. Effect of the dietary fenugreek (*Trigonella foenum-graecum*), used as antioxidant in layer diets, on the nutritional quality of the eggs. Arch. Zootech. 18(2), 45-52.

Park, J.H.A. & Kim, I.H.A., 2015. Interactive effects of fenugreek (*Trigonella foenum - graecum L*) seed extract supplementation and dietary metabolisable energy levels on the growth performance, total tract digestibility, blood profiles, and excreta gas emission in broiler chicken. Anim. Prod. Sci. 56, (10), 1677-1682.

Park, S.-M., Ahn, I.-S., Hong, S.-M., Kim, D.-S., Kwon, D.-Y. & Yang, H.-J., 2010. The effects of the supplementation of *Opuntia humifusa* water extracts and methyl sulfonyl methane on the laying productivity, egg quality and sensory characteristics. J. Korean Soc. Food Sci. Nutr. 39(2), 294-300.

Pelicia, K., Garcia, E., Mori, C., Faitarone, A., Silva, A., Molino, A., Vercese, F. & Berto, D., 2009. Calcium levels and limestone particle size in the diet of commercial layers at the end of the first production cycle. Brazilian J. Poult. Sci. 11, 87-94.

Pelícia, K., Garcia, E. & Scherer, M., 2007. Alternative calcium source effects on commercial egg production and quality. Rev. Bras. 9(2), 105-109.

Petit, P., Sauvaire, Y., Ponsin, G., Manteghetti, M., Fave, A. & Ribes, G., 1993. Effects of a fenugreek seed extract on feeding behaviour in the rat: metabolic-endocrine correlates. Pharmacol. Biochem. Behav. 45, 369-374.

Petrolli, T.G., Albino, L.F.T., Rostagno, H.S., Gomes, P.C., Tavernari, F.C. & Balbino, E.M., 2012. Revista Brasileira de Zootecnia plant extracts in diets for broilers. Rev. Bras. Zootec. 41(7), 1683-1690.

Pizzolante, C.C., Saldanha, E., Laganá, C., Kakimoto, S.K. & Togashi, C.K., 2009. Effects of calcium levels and limestone particle size on the egg quality of semi-heavy layers in their second production cycle. Rev. Bras. Ciência Avícola. 11, 79-86.

Pond, W., Church, D. & Pond, K., 2005. Basic animal nutrition and feeding. 5th ed. New York: John Wiley & Sons.

Prochaska, J.F., Carey, J.B. & Shafer, D.J., 1996. The effect of L-lysine intake on egg component yield and composition in laying hens. Poult. Sci. 75(10), 1268-1277.

Puthpongsiriporn, U., Scheideler, S.E., Sell, J.L. & Beck, M.M., 2001. Effects of vitamin E and C supplementation on performance, in vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress. Poult. Sci. 80, 1190-1200.

Qureshi, S., Banday, M.T., Adil, S., Shakeel, I. & Munshi, Z.H., 2015. Effect of dandelion leaves and fenugreek seeds with or without enzyme addition on performance and blood biochemistry of broiler

chicken, and evaluation of their in vitro antibacterial activity. Indian J. Anim. Sci. 85(11), 1248-1254.

Raju, J., Gupta, D., Rao, A.R., Yadava, P.K. & Baquer, N.Z., 2001. *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Mol. Cell. Biochem. 224(1 2), 45-51.

Reynard, M. & Savory, C.J., 1999. Stress-induced oviposition delays in laying hens: duration and consequences for eggshell quality. Br. Poult. Sci. 40(5), 585591.

Rizzi, C. & Marangon, A., 2012. Quality of organic eggs of hybrid and Italian breed hens C. Poult. Sci. 91, 2330-2340.

Roberts, J.R., 2004. Factors affecting egg internal quality and egg shell quality in laying hens. J. Poult. Sci. 41, 161-177.

Sakomura, N., Basaglia, R., Safortes, C. & Fernandes, J., 2005. Models for estimating metabolizable energy requirements for layer hens. Brazilian J. Animal Sci. 34(2), 575-583.

Samli, H.E., Agma, A. & Senkoylu, N., 2005. Effects of storage time and temperature on egg quality in old laying hens. Poult. Sci. 14(1), 548-553.

SAPA, 2017. Key market signals in the egg industry for the second quarter of 2017. 2Q. 2017. Available at: https://www.sapoultry.co.za/pdf-statistics/egg-industry-key-market-signals.pdf [Accessed 26.11.2018].

Scott, T. & Silversides, F.G., 2000. The effect of storage and strain of hen on egg quality. Poult. Sci. 79(12), 1725-1729.

Shah, M. & Mir, P.S., 2004. Effect of dietary fenugreek seed on dairy cow performance and milk characteristics. Can. J. Anim. Sci. 84(4), 725-729.

Sharma, R., Raghuram, T. & Sudhakar Rao, N., 1990. Effect of fenugreek seeds on blood glucose and serum lipids in Type I diabetes. Eur. J Clin. Nutr. 44(4), 301-306.

Silversides, F.G. & Scott, T.A., 2001. Effect of storage and layer age on quality of eggs from two lines of Hens. Poult. Sci. 80(8), 1240-1245.

Silversides, F.G., Scott, T.A., Korver, D.R., Afsharmanesh, M. & Hruby, M., 2006. A study on the Interaction of xylanase and phytase enzymes in wheat-based diets fed to commercial white and brown egg laying hens Poult. Sci. 85, 297-305.

Singh, Y.N., Devkota, A.K., Sneeden, D.C., Singh, K.K. & Halaweish, F., 2007. Hepatotoxicity potential of Saw Palmetto (*Serenoa repens*) in rats. Phytomedicine. 14(2-3), 204-208.

Smit, H.P.J., 2014. The effect of a natural feed additive, fenugreek, on feed digestibility and milk response in dairy goats (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Sowmya, P. & Rajyalakshmi, P., 1999. Hypocholesterolemic effect of germinated fenugreek seeds in human subjects. Plant Foods Hum. Nutr. 53(4), 359-365.

Świątkiewicz, S., Arczewska-Wlosek, A., Bederska-Lojewska, D. & Jozefiak, D., 2017. Efficacy of dietary vitamin D and its metabolites in poultry - Review and implications of the recent studies. World. Poult. Sci. J. 73(1), 57-68.

Thacker, P.A., 2013. Alternatives to antibiotics as growth promoters for use in swine production: A review. J. Anim. Sci. Biotechnol. 1-12.

Tipu, M., Akhtar, M., Anjum, M.I. & Raja, M., 2006. New dimensions of medicinal plants as animal feed. Pakistan Vet. J. 26(3), 144-148.

Tortuero, F. & Fernandez, E., 1995. Effect of inclusion of microbial culture in barley-based diets fed to layer hens. Anim. Feed Sci. Technology. 53, 255-265.

Travel, A., Nys, Y. and Bain, M., 2011. Effect of hen age, moult, laying environment and egg storage on egg quality. In Improving the Safety and Quality of Eggs and Egg Products: Egg Chemistry, Production and Consumption (pp. 300-329).

Udayasekhara Rao, P., Sesikeran, B., Srinivasa Rao, P., Nadamuni Naidu, A., Vikas Rao, V. & Ramachandran, E.P., 1996. Short term nutritional and safety evaluation of fenugreek. Nutr. Res. 16(9), 1495-1505.

Vondruskova, H., Slamova, R., Trckova, M., Zraly, Z. & Pavlik, I., 2010. Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: A review. Vet. Med. (Praha). 5, 199-224.

Wang, S.B., Jia, Y.H., Wang, L.H., Zhu, F.H. and Lin, Y.T., 2013. Enteromorpha prolifera supplemental level: Effects on laying performance, egg quality, immune function and microflora in feces of laying hens. Chin. J. Anim. Nutr., 25, pp.1346-1352.

Whitehead, C.C., 2004. Overview of bone biology in the egg-laying hen. Poult. Sci. 83(2), 193-199.

Zaghini, A., Martelli, G., Roncada, P., Simioli, M. & Rizzi, L., 2005. Mannanoligosaccharides and aflatoxin B1 in feed for laying hens: effects on egg quality, aflatoxins B1 and M1 residues in eggs, and aflatoxin B1 levels in liver. Poult. Sci. 84, 825-832.

Zaheer, K., 2015. An updated review on chicken eggs: Production, consumption, management aspects and nutritional benefits to human health. Food Nutr. Sci. 6, (October), 1208-1220.

Zhang, J.W., Chen, D.W., Yu, B. & Wang, Y.M., 2011. Effect of dietary energy source on deposition and fatty acid synthesis in the liver of the laying hen. Br. Poult. Sci. 52(6), 704-710.

Zhang, P., Yan, T., Wang, X., Kuang, S., Xiao, Y., Lu, W. & Bi, D., 2017. Probiotic mixture ameliorates heat stress of laying hens by enhancing intestinal barrier function and improving gut microbiota. Ital. J. Anim. Sci. 16(2), 292-300.

Chapter 3: Production Parameters

Abstract

Two natural feed additives (Nutrifen® and Nutrifen Plus®) were used in the diets of 100 Hy-Line layer hens (32 weeks old) to determine their effects on the total feed intake, egg number and egg weight, energy intake, lysine intake, protein intake, body weight and feed conversion ratio (FCR) of the hens. The hens were divided equally into five treatment groups (i.e., 20 layers per group) and housed randomly in individual cages. The first treatment group received a basal diet (control), while the other four treatment groups received the basal diet supplemented with different percentages (0.1% or 0.2%) of the feed additive: Nutrifen® 0.1% (N1), Nutrifen® 0.2% (N2), Nutrifen Plus® 0.1% (N+1) and Nutrifen Plus® 0.2% (N+2). The layers were fed with the treatment diets for one month. The results recorded showed no significant difference between all the treatments and the control group, on all the parameters evaluated. However, significant differences were found between the control and all four other treatment diets (N1, N2, N+1 and N+2) for egg number. The overall effect of fenugreek on the other measured production parameters was therefore insignificant and it is concluded that the addition of fenugreek had not effect.

Keywords: egg weight; fenugreek; feed additives; intake

3.1 Introduction

The aim of a layer hen production system is to obtain optimum production at the lowest cost (Lacin *et al.*, 2008). However, several production parameters, such as egg number and egg weight, body weight, nutrient intake and the feed conversion ratio (FCR) need to be maintained to achieve optimum and cost-effective production. These egg production parameters are highly affected by the diet of the hen. In a balanced diet, the addition of feed additives may positively contribute to the improvement and maintenance of egg production. The ban on antibiotic feed additives in Europe initiated investigations into natural feed additives as alternatives. A variety of natural feed additives have been tested to determine their effect on egg production, however, limited evidence exists to express their exact effects on egg production and quality specifically. This chapter focuses solely on fenugreek as a natural feed additive in a layer hen diet and its effects on egg production.

The seeds from the fenugreek plant have a variety of effects on the production of layer hens. These include improved body weight of layers (Awadein *et al.*, 2010) and broilers (Abaza, 2001), enhanced digestibility of feed, increased feed consumption and improved FCR (Abaza, 2007). The latter

may lead to an increase in feed cost, however, the feed cost per unit production will be lower and therefore result in an overall improvement of production (Moustafa, 2006). In addition, fenugreek has been used to improve the intestinal health of hens.

Natural feed additives have been used beneficially in the diets of layer hens to improve their gut health, digestibility and nutrient absorption, which ultimately increases productivity (Adil *et al.*, 2015). Fenugreek reduces the lipid content of the liver (Awadein *et al.*, 2010), its active ingredients have a hepato-protective action and it has antioxidant capacity (Bukhari *et al.*, 2008) which all contributes to improving the health status of the hen. An increase in the villus height and width (through reduced inflammatory reaction at the mucosa) (Loddi *et al.*, 2004; Mahmood *et al.*, 2015) and an increase in the crypt depth and surface area has been reported on the inside of small intestine as a result of fenugreek being used as a feed additive in layer hens (Abdel-Rahman *et al.*, 2014). This results in improved intestinal health (Petrolli *et al.*, 2012), absorption and nutrient utilisation (Adil *et al.*, 2010), which improves the hen's performance (Samanya and Yamauchi, 2002). The improved performance as a result of improved intestinal health in turn leads to improved egg production qualities. As a result, egg production and egg weight were also found to be increased with the dietary supplementation of fenugreek (Hassan & Ragab, 2001). In order for fenugreek to exert these positive effects, however, fenugreek should be added to the diet at the right level and concentration.

Feed additives, which are usually very concentrated, are added to the diet in small percentages of the total diet. The inclusion levels of fenugreek may also have an effect on egg production. The supply of excess fenugreek may result in wastage as the animal may not be able to utilise the high supply. Consequently, this may result in losses due to the cost of inclusion of the product in the diet being higher than necessary. On the contrary, low inclusion levels may have little to no effect on egg production due to the supply not being sufficient to stimulate a response. Therefore, including fenugreek at the right inclusion level is important. While Abbas (2010) suggests that an inclusion level of 0.5% fenugreek yields the best results in broiler chickens, Mamoun *et al.* (2014) report that the best results are achieved at an inclusion level of 1% in terms of body weight gain, FCR, protein efficiency ratio, feed intake and efficacy of energy utilisation.

Currently, there is limited research available regarding the use of Nutrifen® and Nutrifen Plus® (fenugreek products) on the production parameters of Hy-Line layer hens. Since an increase in egg weight, egg number per time unit and FCR will increase economic gain and farm productivity, it is important to investigate the effect of Nutrifen® and Nutrifen Plus® on these production parameters to determine its validity as a beneficial natural feed additive for layer hens.

3.2 Materials and methods

Ethical clearance for this trial was obtained from the ethical committee of Stellenbosch University (SU-ACUD16-000099). The experimental techniques and practices were in accordance with the regulations set out by the ethical committee and the code of conduct of the South African Poultry Association (SAPA).

3.2.1 Animals, experimental design and management

For this study, 100, 32-week-old Hy-Line brown layer hens were housed at Stellenbosch University's Mariendahl Experimental farm (Poultry division). The birds were housed in a naturally ventilated house and were exposed to light (16 hours per day) and dark hours (8 hours per day) controlled by a timer. On very hot days (>25ºC), water sprayers (located on the roof) were used to cool down the house and the hens. The hens were divided randomly into five treatment groups of 20 birds per group, and placed into individual cages (approximately 1^2 meter in size). Each cage was fitted with a water nipple (drinker) and connected to a feeding trough. The birds were examined daily for any signs of distress and illness.

3.2.2 Temperature

The environmental temperature and house temperature may affect the intake and production of the layers. The temperature for the duration of the trial is reported in Figure 3.1. All the birds were exposed to the same temperatures at all times; therefore their reaction to the environment will be similar. No extreme temperature changes were observed for the duration of the trial.

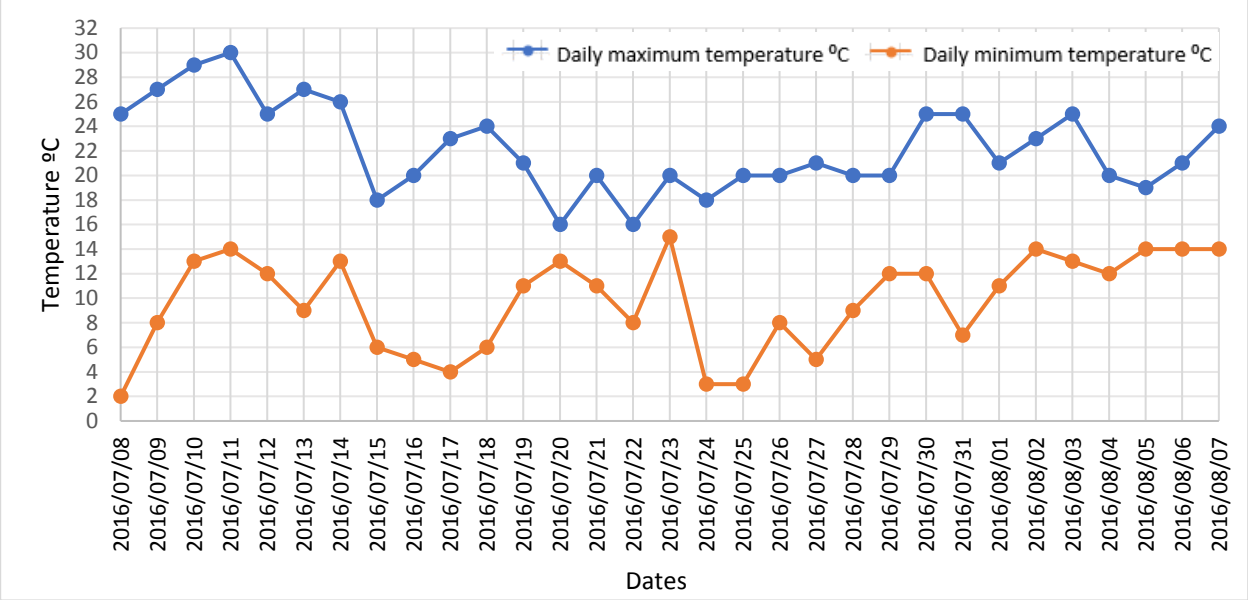

Figure 3.1 Daily maximum and minimum temperatures of the layer house for the duration of the trial

3.2.3 Water and diet

The water drinkers of each cage were checked daily to ensure an adequate water supply to each bird. During an adaptation phase of two weeks, the birds were fed a standard layer hen diet followed by the treatment diets. The five treatments were as follows: Control; 0.1% Nutrifen® (N1); 0.2% Nutrifen® (N2); 0.1% Nutrifen Plus® (N+1); and 0.2% Nutrifen Plus® (N+2). The inclusion levels of Nutrifen® and Nutrifen Plus® were stipulated by the manufacturers as 0.1% and 0.2% due to the major ingredient being the fenugreek seed extract, which is highly concentrated compared to other parts of the plant. Due to limited knowledge about the heat stability of Nutrifen® and Nutrifen Plus®, the diets were fed in a mash form to avoid possible heat damage.

A standard layer diet (basal) was formulated using Winfeed (version 1.13), in which a filler was included to substitute for the addition of the feed additive Nutrifen® and Nutrifen Plus®. The five diets were mixed at the Mariendahl experimental farm with an industry standard blender/mixer (10 kg). A standard layer diet made up of the ingredients (as indicated in the first column of

Table 3.1) was mixed first, after which the Nutrifen® and then Nutrifen Plus® diets were mixed in increasing levels of concentration (lower concentration mixed first). The blender was cleaned between each diet mixture. All the raw materials were weighed carefully and mixed thoroughly to form a meal. The meal was stored in a cool dry place in 10 kg plastic bags (which are easy to handle and carry) until

it was used for the start of the adaption period of the trial. A sample (200 g) of each experimental diet was weighed out before the diets were fed and was stored in a refrigerator for proximate analysis. The samples were milled with a 1 mm screen and analysed for ash, dry matter, crude protein, crude fibre and crude fat content (Section 3.2.5 Proximate analysis).

Table 3.1 The composition of ingredients of the basal diet and the actual calculated nutrient composition of the diet

Ingredients	**Unit**	**Control**
Maize	%	69.821
Soybean full fat	%	1.332
Soybean 46	%	18.018
DL methionine	%	0.090
Vitamin + mineral premix	%	0.250
Filler	%	0.200
Limestone	%	8.056
Salt	%	0.147
Monocalcium phosphate	%	1.691
Sodium bicarbonate	%	0.394
Nutrifen®	%	0.000
Nutrifen Plus®	%	0.000
Calculated nutritional vales		
[1]AME_n	MJ/kg	11.30
Dry matter	g/kg	899.99
Moisture	g/kg	100.01
Crude protein	g/kg	131.25
Crude fat	g/kg	29.61
Crude fibre	g/kg	25.68
Ash	g/kg	100.36
Lysine	%	0.727

3.2.4 Measuring and sampling

The layer hens (n = 100) were weighed prior to the start of the adaption period, on the first day of of the trial (day 1) and at the end of the trial (day 31). The minimum and maximum temperatures in the house were recorded daily. Each hen received a daily portion of 180 g of their respective experimental diets and the daily feed intake was monitored. The feed supply was increased (and recorded) for hens

who finished all the given feed within 24 hours. The remaining feed of each hen was collected, removed and weighed every 5 days for the duration of the trial. This data was used to calculate the actual nutrient intake of birds on the respective diets (

Table 3.1).

Eggs were collected daily at approximately mid-day (12:00). Each egg was marked (using a pencil) with the hen's number and date, and then placed in egg boxes. The egg boxes were marked with dates, hen numbers and treatment for easy identification. On the day of collection the eggs were weighed, counted and stored in a laboratory room at room temperature (15 ºC to 17 ºC) prior to the egg quality analysis. Egg quality analysis was performed on the eggs from six selected hens per treatment who laid an egg every day for the duration of the trial.

The data collected from the proximate analysis and feed formulation of each trial was used to calculate the FCR for egg production and the remaining parameters as indicated below (Equation 3.1 to Equation 3.8).

Equation 3.1:

FCR (per kg egg mass) = (kg of feed consumed) / (kg of eggs produced)

Equation 3.2:

Average feed intake per egg laid (g) = (Total intake) / (Total number of eggs)

Equation 3.3:

Lysine consumed per gram of egg (g) = (Total lysine consumed) / (Total egg weight)

Equation 3.4:

Average lysine consumed per egg laid (g) = (Total lysine consumed) / (Total number of eggs)

Equation 3.5:

Energy consumed per gram of egg (MJ/g) = (Total energy consumed) / (Total egg weight)

Equation 3.6:

Average energy consumed per egg laid (MJ/g) = (Total energy consumed) / (Total number of eggs)

Equation 3.7:

Protein consumed per gram of egg (g) = (Total protein intake) / (Total egg weight)

Equation 3.8:

Average protein consumed per egg laid (g) = (Total protein intake) / (Total number of eggs)

3.2.5 Proximate analysis

Dry matter

The dry matter (DM) of feed samples was determined using the methods as described by the Association of Official Analytical Chemists (AOAC, 2002), official method 934.01. Feed samples were analysed in duplicate, where 2 g of each sample was weighed into a pre-weighed porcelain crucible and then dried at 100°C for 24 hours. Thereafter, the dried samples were weighed, and the DM content calculated using

Equation 3.9.

Equation 3.9:

$$\%\text{Moisture} = \frac{(A+B) - C}{B} \times 100$$

$$\%\text{DM} = 100 - \%\text{Moisture}$$

where:

A = weight of empty crucible

B = weight of air dried sample

C = weight of crucible and dry sample

Ash content

The samples retained from the dry matter analysis were used to determine the ash content of the feed. Ash content was determined as described by the AOAC (2002), official method 942.05. Feed samples were combusted in a combustion oven at 500°C for six hours. Thereafter, the combusted samples were cooled in a desiccator before being weighed, and the ash content calculated using Equation 3.10.

Equation 3.10:

$$\%\text{Ash} = \frac{D - A}{\text{sample mass}} \times 100$$

$$\%\text{Organic matter} = 100 - \%\text{Ash}$$

where:

A = weight of empty and dry crucible

D= weight of crucible and ash

Crude protein

The official method 4.2.07, as described by the AOAC (2002), was used to determine the crude protein (CP) content of all samples by measuring the total nitrogen (N) content in a LECO FP528 apparatus. Feed samples were analysed in duplicate, where 1 g of each sample was weighed in a foil cup and then placed into the LECO FP528. The N content was directly recorded from the measurement of the LECO FP528, and the CP content was calculated using Equation 3.11.

Equation 3.11:

$$CP(\%) = N(\%) \times 6.25$$

Crude fibre

The crude fibre content of the feed was determined using the Filter Bag Technique and an ANKOM Fibre Analyser. A sulphuric acid (H_2SO_4) solution (0.255 N) and a sodium hydroxide (NaOH) solution (0.313 N) were used as reagents. Approximately 1 g of sample was weighed into a pre-weighed ANKOM filter bag and heat-sealed. Fat was then extracted from the samples by soaking the bags in petroleum ether for 10 minutes, after which the bags were air-dried. Bags were then placed in the ANKOM fibre analyser and agitated at 100ºC in 1.9 L of the H_2SO_4 solution for 40 minutes. After the first extractions were completed, the samples were rinsed twice with hot water in the fibre analyser machine. Samples were then agitated at 100ºC in 1.9 L of the NaOH solution for 40 minutes. Thereafter, samples were rinsed three times with hot water to complete the ANKOM analysis. The samples were then removed and air-dried, soaked in acetone for 5 minutes, and once again air dried before being placed in the 100ºC oven to dry for 2 to 4 hours. Once the samples were removed from the oven and left to cool, they were each weighed and placed in a pre-weighed porcelain crucible. Samples were then incinerated in the crucibles at 500°C for 5 hours. The ashed samples were weighed and the percentage crude fibre determined using Equation 3.12. A single blank bag containing no sample was also included into each run to determine the blank bag correction factor.

Equation 3.12:

$$\%\text{Crude fibre} = 100 \times \frac{W_3 - (W_1 \times C_1)}{W_2}$$

where:

W_1 = bag tare weight

W_2 = sample weight

W_3 = weight of organic matter (loss of weight on ignition of bag and fibre)

C_1 = Ash corrected blank bag factor (loss of weight on ignition of blank bag / original blank bag).

Crude fat

The crude fat (CF) or ether extract (EE) content was determined by making use of the diethyl ether reagent method using a Tecator Soxtec System HT 1043 Extraction Unit according to the AOAC (2002), official method 920.39. Duplicates of each sample were analysed, and a 2 g sample of feed was weighed and placed in a thimble. Thereafter, 50 ml of diethyl ether was added to a pre-weighed soxhlet beaker and placed into the Tecator Soxtec System HT 1043 Extraction Unit. After the fat extraction into the soxhlet beaker, the beaker was placed in a drying oven for 2 hours at 100ºC and weighed again. The CF content was then calculated using Equation 3.13.

Equation 3.13:

$$\text{Crude fat \%} = \frac{(\text{Mass of soxhlet cup + fat}) - (\text{mass of soxhlet cup})}{\text{Mass of sample}} \times \frac{100}{1}$$

Equation 3.14:

Average lysine intake = (Total feed intake) x (Percentage calculated lysine in feed)

3.3 Statistical analysis

The Hypotheses tested:

H_0: There is no relationship between the production parameters and the treatment diets

H_a: There is evidence to suggest that there is a relationship between the production parameters and the treatment diets

The experiment was based on a completely randomised design. The data was analysed using a mixed model analysis of variance (ANOVA), with the treatment diets and the production parameters tested as main effects, using Statistica 64 (Version 13.2, 2017). The significant differences between means were tested using least significant difference (LSD) tests and a Fisher's LSD post hoc test was used to identify individual differences observed between treatments. A probability value of $P \leq 0.05$ indicated that the difference between treatments were statistically significant (i.e., 5% level of significance).

3.4 Results and discussion

3.4.1 Mean daily feed intake of hens

No significant differences were observed between treatments for the five-day feed intake and the overall feed intake. This indicates that Nutrifen® and NutrifenPlus® had no effect on the feed intake of the birds throughout the trial period. The effect of Nutrifen® and NutrifenPlus® on feed intake is discussed in more detail in Section 3.4.3 Production parameters.

Table 3.2 Average (± standard deviation) five daily and average daily feed intake (g) of layers fed one of five diets over a period of one month

Treatment	Five day intake (g)						Avg. Intake	Daily
	Day 0-5	Day 6-10	Day 11-15	Day 16-20	Day 21-25	Day 26-30		
Control	693 ± 13.34	698 ± 13.34	686 ± 13.34	666 ± 13.34	636 ± 13.34	641 ± 13.34	669 ± 10.55	134
Nutrifen® 0.1%	698 ± 12.66	690 ± 12.66	645 ± 12.66	651 ± 12.66	653 ± 12.66	645 ± 12.66	664 ± 10.01	133
Nutrifen® 0.2%	719 ± 12.66	716 ± 12.66	675 ± 12.66	649 ± 12.66	668 ± 12.66	678 ± 12.66	684 ± 10.01	137
Nutrifen Plus® 0.1%	739 ± 12.66	726 ± 12.66	673 ± 12.66	675 ± 12.66	665 ± 12.66	674 ± 12.66	692 ± 10.01	138
Nutrifen Plus® 0.2%	742 ± 12.66	711 ± 12.66	669 ± 12.66	676 ± 12.66	686 ± 12.66	676 ± 12.66	693 ± 10.01	139
P values	0.28	0.40	0.46	0.50	0.14	0.26	0.15	

3.4.2 Live weight

The average initial body weight and the average body weight gain of the hens for the full duration of the trial is shown in Table 3.3. The control diet, Nutrifen® 0.1% (N1) and Nutrifen Plus® 0.1% (N+1) showed no significant differences between each other for the average body weight gain for the duration for the experiment. The Nutrifen® 0.2% (N2) diet resulted in a significantly greater body weight gain compared to all other treatments. Whilst, Nutrifen Plus® 0.2% (N+2) resulted in a significantly lower body weight gain compared to all the other treatment diets.

The non-significant difference found in average body weight gained between the control, N1 and N+1 is supported by the results from Abaza (2007) and Safaa (2007). They both observed no significant differences in body weight gain for diets including Nutrifen® at 0.5% (Abaza, 2007) and 2% fenugreek (Safaa, 2007), compared to a control diet. The inclusion levels are all different and therefore this can be

eliminated as a possible reason for the non-significant differences observed between the control, N1 and N+1 treatments of this diet. The differences in treatment diets of Nutrifen® and NutrifenPlus® can also be excluded as both these treatments performed similar to that of the control diet at a 1% inclusion level.

In addition, the significant higher body weight gain achieved by birds on the N2 diet is in line with the results observed from Awadein *et al.* (2010), who also found a significant increase in live body weight of hens fed 0.1% and 0.5% fenugreek compared to a control group. Moustafa (2006) also observed similar results at an inclusion level of only 0.05% fenugreek. Murray *et al.* (1991) reported that the presence of essential fatty acids and high-quality protein of fenugreek may be responsible for the improvement in body weight gain. However, this assumption cannot be applied to the current study due to the low inclusion levels (i.e., 0.1% and 0.2%) that were used, which resulted in all the diets having a similar protein content (

Table 3.1). On the other hand, Elagib *et al.* (2013) and Hernández *et al.* (2004) reported that the increase in body weight may be due to the ability of fenugreek to stimulate the digestive system.

A significantly lower weight gain was observed by the hens on the N+2 diet compared to the control and the other treatments and may be due to a reaction of the birds on the higher levels and the active ingredients of NutrifenPlus®. In the N+2, MSM is also included, which is also a growth promoting additive. Therefore this result is unconventional. There is however little evidence to no evidence about the interaction between MSM and fenugreek.

Hen body weight significantly impacts on egg production, therefore flock uniformity in the beginning and during production is important. Also, egg numbers decreases while egg weight and feed consumption increase with an increase in body weight. This is due to heavier birds having a higher maintenance requirement and therefore eating more and laying eggs with larger egg yolks compared to lighter hens. A slower body weight gain during production may therefore be beneficial to reduce the cost of maintenance (Leeson *et al.*, 1996). However, eggs are the marketable product and an increase in egg weight and yolk size may be more beneficial in terms of profit. The ratio between the cost of maintenance and egg price needs to be calculated carefully to determine the profit margin.

Table 3.3 The mean (± standard deviation) of the initial weight, end weight and weight gain of the hens receiving either Nutrifen® or NutrifenPlus® supplemented diets at various inclusion levels during a 30 day feeding trial

Treatment (diet)	Average body weight (pre-adaption) (kg)	Average initial body weight (kg)	Average final body weight (kg)	Average weight gain (kg)
Control	1.86 ± 0.208	1.86 ± 0.172	1.97 ± 0.185	0.11^{a} ± 0.013
Nutrifen® 0.1%	1.82 ± 0.180	1.82 ± 0.145	1.93 ± 0.133	0.10^{a} ± 0.012
Nutrifen® 0.2%	1.86 ± 0.174	1.87 ± 0.148	1.99 ± 0.166	0.13^{b} ± 0.012
Nutrifen Plus® 0.1%	1.86 ± 0.178	1.87 ± 0.143	1.97 ± 0.138	0.10^{a} ± 0.012
Nutrifen Plus® 0.2%	1.84 ± 0.144	1.87 ± 0.142	1.94 ± 0.135	0.07^{c} ± 0.012
P values	0.94	0.85	0.65	0.03

($^{a, b,c,}$) Means with different superscripts within the same column differ significantly ($P \leq 0.05$).

3.4.3 Production parameters

The means (± standard error) for all the production parameters are presented in Table 3.4. There were no significant ($P > 0.05$) differences between treatments in any of the parameters measured except for egg number.

The results observed for feed intake showed no significant ($P > 0.05$) differences between any of the treatments. This is in accordance with the findings of Moustafa (2006), who investigated an inclusion of fenugreek at 0.05%, 0.1% and 0.15%, and found no significant difference in intake compared to the control group. El-Kaiaty *et al.* (2002) also reported no improved effect of fenugreek on feed intake. In contradiction to the above-mentioned studies and the study at hand, Abaza (2007) found that the feed intake was lower (compared to the control) for fenugreek-fed hens at a 0.5% inclusion level. This inclusion level is much higher than the current study and may have affected the palatability of the feed. Fenugreek has a very strong flavour, which could affect feed intake when given in large amounts (Eneyew, 2006). In broilers, Alloui *et al.* (2012) concluded that the addition of 0.3% fenugreek will significantly increase the feed intake. The presence of galactomannans and neurin in fenugreek positively stimulate gut micro flora and may be responsible for stimulating appetite and thereby improving feed intake and thus the FCR (Alloui *et al.*, 2012). Yet, these results may differ from the current study due to the different basal diets and the different production needs that exist between broilers and layers. In addition, the differences could be explained by the differences in the inclusion levels. The 0.3% inclusion level may therefore have been more effective than lower inclusion levels of 0.1 and 0.2%.

Table 3.4 Mean (± standard error) and *P* values for the production parameters of Hy-Line layer hens receiving diets containing no additive, Nutrifen® or NutrifenPlus® at various inclusion levels

Parameter	Treatment (diet)					*P* value
	Control	**Nutrifen® 0.1%**	**Nutrifen® 0.2%**	**Nutrifen Plus® 0.1%**	**Nutrifen Plus® 0.2%**	
Total feed intake (g)	3928 ± 79.5	3964 ± 79.5	4013 ± 79.5	4220 ± 79.5	4200 ± 83.1	0.11
Total egg weight (g)	1679 ± 39.7	1690 ± 39.7	1657 ± 39.7	1704 ± 39.7	1717 ± 41.4	0.86
Average egg number	28^{a} ± 0.131	30^{b} ± 0.164	30^{b} ± 0.190	30^{b} ± 0.152	30^{b} ± 0.154	0.00
Total energy intake (MJ)	44.39 ± 8.987	44.79 ± 8.987	45.35 ± 8.987	47.68 ± 8.987	47.46 ± 9.386	0.11
Total lysine intake (g)	29.98 ± 0.578	28.83 ± 0.578	29.18 ± 0.578	30.68 ± 0.578	30.54 ± 0.604	0.11
Total protein intake (g)	515.6 ± 11.40	521.1 ± 11.40	529.8 ± 11.40	555.2 ± 11.40	555.2 ± 11.90	0.50
Feed conversion ratio	2.34 ± 0.073	2.35 ± 0.073	2.47 ± 0.073	2.48 ± 0.073	2.45 ± 0.076	0.70
Feed intake/ egg (g)	140.3 ± 3.46	132.13 ± 3.46	133.8 ± 3.46	140.7 ± 3.46	140.0 ± 3.62	0.14
Lysine intake/kg egg (g/kg)	17.86 ± 0.100	17.06 ± 0.100	17.61 ± 0.100	18.00 ± 0.100	17.79 ± 0.100	0.70
Lysine intake/egg	1.07 ± 0.025	0.96 ± 0.025	0.97 ± 0.025	1.01 ± 0.025	1.01 ± 0.026	0.54
Energy intake/kg egg (MJ/kg)	26.44 ± 0.821	26.50 ± 0.821	27.37 ± 0.821	27.98 ± 0.821	27.64 ± 0.858	0.70
Energy intake/egg (MJ)	1.59 ± 0.039	1.49 ± 0.039	1.51 ± 0.039	1.59 ± 0.039	1.58 ± 0.041	0.54
Protein intake/ kg egg (g/kg)	0.35 ± 0.010	0.34 ± 0.010	0.36 ± 0.010	0.35 ± 0.010	0.35 ± 0.011	0.78
Protein intake/egg (g)	18.41 ± 0.499	17.39 ± 0.499	17.53 ± 0.499	18.51 ± 0.499	18.51 ± 0.521	0.66

($^{a, b,}$) Means with different superscripts within the same column differ significantly ($P \leq 0.05$).

No significant differences (P >0.05) were found between the treatments for FCR (Table 3.4). Abaza (2007) also studied layer hens from 32-39 weeks of age and observed no changes in the FCR of layers that were fed a diet supplemented with 0.5% fenugreek, compared to the control group. Although this inclusion level is much higher than the current study, it has rendered a similar response in terms of FCR. In contrast to the results of this study, other experiments (Moustafa, 2006; Abaza, 2007) with both high (0.5%) and low (0.1%) inclusion levels, have found significant improvement in the FCR of older layer hens (40-59 and 40-43 weeks, respectively). It could be that at this older age, the birds are under reproductive stress and the presence of fenugreek in the diet assisted in maintaining the FCR while stress was experienced. It should also be noted that the fenugreek products used in the different experiments are not the same; there may be differences in the active ingredients. The active ingredients of Nutrifen Plus® differs from that of Nutrifen® and differences also exist between other fenugreek products; therefore, the differences observed in experiments may be due to the differences in active ingredients of the products used. In addition, the differences in the basal diets between experiments may also be responsible for the different results obtained. Fenugreek also contains flavonoids known to have antibacterial activity, which improves the number of beneficial intestinal flora and balance of metabolites (Bhatti *et al.*, 1996). The latter positively affects gastrointestinal tract micro-organisms, resulting in improved FCR (Abdalla *et al.*, 2011).

A significant difference (P <0.05) was found between the average amount of eggs laid by hens from the respective treatment groups. The control diet group laid a significantly lower (28) amount of eggs for the trial period compared to the four other treatment groups whom all laid 30 eggs on average. In contradiction to these results, Abaza (2007) found that a 0.5% inclusion of fenugreek had no significant effect the number of eggs produced. At a 2% inclusion, El-Kaiaty et al. (2002), Tollba et al. (2005) and Safaa (2007) also found that fenugreek had no significant effect on egg number.

In addition, the results show no significant difference (P >0.05) in egg weight between treatments (Table 3.4). The significant increase in body weight gain for N2 and loss for N+2 as indicated in section 3.4.2 therefore did not have an effect on the overall total egg weight between treatments. In support of these results, Abaza (2007) found that the inclusion of fenugreek at a 0.5% level had no significant effect on egg weight nor the number of eggs produced. El-Kaiaty *et al.* (2002), Tollba *et al.* (2005) and Safaa (2007) also found that fenugreek had no significant effect on egg number and egg weight when included in the diet at an inclusion level of 2%. There are significant differences in the inclusion levels of these diets mentioned compared to the present

study, yet similar results were observed. This may indicate that fenugreek has little effect on egg weight and the number of eggs produced, despite the inclusion level.

However, many researchers (Hassan & Ragab, 2001; Abdalla *et al.*, 2011; Alobaidy, 2012) have found a significant effect of fenugreek-supplemented diets on egg production and egg weight. Alobaidy (2012) reported an improvement in layer hen reproductive performance with the inclusion of fenugreek (0.3%) in the diet. Hassan & Ragab (2001) found significant improvement in egg weight, egg number and egg quality. At an inclusion level of 2%, Abdalla *et al.* (2011) found no significant changes in egg weight for the first period (29-40 weeks of age). However, at a later period (41-48 weeks of age), the egg weight of the fenugreek-fed hens increased significantly compared to that of the control. The results obtained in the above-mentioned studies (which differed from the results of the current study) regarding egg weight and egg number may have been influenced by the hens' age. Hens experience physiological stress in the later stage of the production cycle. Older hens have a reduced ability to absorb calcium from the bone and produce less calcium carbonate to deposit into the egg shell. Calcium absorption from the feed and the mobilisation of calcium also decreases significantly in older hens (Mahmoud *et al.*, 1994). This ultimately results in thinner egg shells and larger, heavier eggs. The hens in this study were not under physiological stress where hen age is concerned; this can therefore explain the differences in terms of egg weight being consistent for all treatments.

Energy intake did not differ (P >0.05) significantly between treatments for this study. This was expected as the energy levels of all the treatment diets and the control diet was consistent. When a diet is fed *ad libitum*, the hen will continue to eat until she satisfies her energy requirements. Due to the similar dietary energy levels in all the diets and the birds being uniform, the intake of energy should be consistent across groups (National Research Council US, 1984).

In addition, no significant difference (P >0.05) was found for the CP intake of hens among all the treatments and the control (Table 3.4). Therefore, no significant differences (P >0.05) were found between treatments for the amount of protein consumed per egg produced and per gram of egg weight. Protein intake is dependent on the actual intake of the complete diet. Protein is received by the hen through amino acids supplied. The essential amino acids need to be provided through the diet, while non-essential amino acids can be synthesised if enough protein and essential amino acids are available (National Research Council US, 1984).

Methionine is the first limiting amino acid, which means its requirements can only be met through the supply of methionine. A deficiency in dietary methionine can lead to reduced growth

and a decrease in disease resistance as methionine is a methyl donor for cellular metabolism (Bunchasak, 2009). Lysine is the second limiting amino acid and its requirement is based on that of methionine. Methionine and lysine are thus reference amino acids by which the ratio of all other amino acids is calculated (Farkhoy *et al.*, 2012). The intake of the first limiting amino acids is important for protein synthesis. When these limiting amino acids are reduced, production is affected.

The amount of feed, lysine, protein and energy consumed to produce a single egg did not differ significantly (P >0.05) between treatments (Table 3.4). Similarly, the lysine, protein and energy consumed to produce one gram of egg did not differ significantly (P >0.05) between treatments. These results were expected, given that there was no significant difference between treatments for egg weight (which are used to calculate the above-mentioned parameters). Prochaska *et al.* (1996) also found no effect of increasing lysine concentrations on egg weight, while Rama Rao *et al.* (2011) reported a non-linear increase in egg mass with increasing dietary lysine concentrations. However, Novak and Scheideler (1998) found that lysine had no effect on the egg weight during the first period (20-43 weeks) but caused a significant difference in egg weight during the second period (44-63 weeks); this indicates that lysine from the diet plays a greater role in the egg weight of older hens.

3.5 Conclusion

A positive effect of both Nutrifen® and Nutrifen Plus® (0.1% and 0.2%) on egg production (number) was evident in this study. This response will contribute to the productivity of a layer farm. The rest of the results of this study indicate that Nutrifen® and Nutrifen Plus® at the respective inclusion levels (0.1% and 0.2%) had no positive nor negative effect on the production parameters studied: total intake (g), egg weight, energy intake (MJ/kg), lysine intake (g), protein intake (g) and the FCR. The low inclusion levels and no stress experienced are two of the main possible reasons for the results observed. The study did however show significant differences in live body weight of hens on the N2 and N+2 diets. The N2 diet had a significantly higher body weight gain compared to all other treatments, while the N+2 diet exerted the opposite effect by having a significantly lower body weight gain compared to the other treatments. The changes observed in body weight gain had no effect on any other production parameters tested. A conclusion can therefore be made for this study that Nutrifen® at an inclusion level of 0.2% significantly increases body weight gain, while 0.2% Nutrifen Plus® significantly decreases body weight gain.

A variety of inclusion levels of the product can be tested to observe which inclusion level achieves optimum results for the parameters studied. In addition, the application of stressors such as age differences or physiological stress and environmental stress may also render different outcomes. Nutrifen® and Nutrifen Plus® may contribute to maintaining production during stress. This can be investigated for future research into these specific products.

3.6 References

Abaza, I.M., 2001. The use of some medicinal plants as feed additive in broiler diets. Ph.D. Thesis, Faculty of Agriculture, Alexandria University, Egypt.

Abaza, I.M., 2007. Effects of using fenugreek, chamomile and radish as feed additives on productive performance and digestibility coefficients of laying hens. Egypt. Poult. Sci. 27, 199-218.

Abbas, R.J., 2010. Effect of using fenugreek, parsley and sweet basil seeds as feed additives on the performance of broiler chickens. Int. J. Poult. Sci. 9(3), 278-282.

Abd El-Aal, M.H. & Rahma, E.H., 1986. Changes in gross chemical composition with emphasis on lipid and protein fractions during germination of fenugreek seeds. Food. Chem. 22(3), 193-207.

Abdalla, A.A., Ahmed, M. & Abaza, I.M., 2011. Effect of using some medicinal plants and their mixtures on productive and reproductive performance of gimmizah strain 2-egg production period. Egypt. Poult. Sci. 31(2), 641-654.

Abdel-Rahman, H.A., Fathallah, S.I., Helal, M.A., Nafeaa, A.A. & Zahran, I.S., 2014. Effect of turmeric (*curcuma longa),* fenugreek (*trigonella foenum-graecum L*.) and/or bioflavonoid supplementation to the broiler chicks' diet and drinking water on the growth performance and intestinal morphometeric parameters. Glob. Vet. 12(5), 627-635.

Adil, S., Banday, T., Bhat, G.A., Mir, M.S. and Rehman, M., 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. *Veterinary medicine international*, *2010*.

Adil, S., Qureshi, S. & Pattoo, R., 2015. Review on positive effects of fenugreek as poultry feed additive. Int. J. Poult. Sci. 14(12), 664-669.

Alloui, N., Ben-Aksa, S., Alloui, M. & Ibrir, F., 2012. Utilization of fenugreek (*Trigonella Foenum-Graecum*). J. World. Poult. Res. 2(2), 25-27.

Alobaidy, R., 2012. Effect of fenugreek seeds and olive leaves ration supplementation on productive and physiological performance of laying breeder hen. MSc thesis, College of Agriculture and Forestry, University of Mosul, Iraq, pp. 1-40.

AOAC, 2002 Official method of analysis. 16th ed. Association of Official Analytical, Washington DC.

Awadein, N.B., Eid, Y.Z. & Abdel-Ghany, F.A., 2010. Effect of dietary supplementation with phytoestrogens sources before sexual maturity on productive performance of mandarah hens. Egypt. Poult. Sci. 30(3), 829-846.

Balnave, D. & Muheereza, S.K., 1997. Improving eggshell quality at high temperatures with dietary sodium bicarbonate. Poult. Sci. 76(4), 588-93.

Bhatti, A., 1996. Antibacterial activity of *trigonella foenum-graecum* seeds. Phytotherapeu. 67, 372-374.

Bordas, A., Tixier-Boichard, M. & Merat, P., 1992. Direct and correlated responses to divergent selection for residual food intake in Rhode Island red laying hens. Br. Poult. Sci. 33(4), 741-754.

Bukhari, S.B., Bhanger, M.I. & Memon, S., 2008. Antioxidative activity of extracts from fenugreek seeds (*Trigonella foenum-graecum*). J. Anal. Environ. Chem. 9(2), 78-83.

Bunchasak, C., 2009. Role of dietary methionine in poultry production. The Journal of Poult. Sci. 46, 169-179.

Duan, X., Li, F., Mou, S., Feng, J., Liu, P. & Xu, L., 2015. Effects of dietary L-arginine on laying performance and anti-oxidant capacity of broiler breeder hens, eggs, and offspring during the late laying period. Poult. Sci. 94, 2938-2943.

Elagib, H.A.A., Abbas, S.A. & Elamin, K.M., 2013. Effect of different natural feed additives compared to antibiotic on performance of broiler chicks under high temperature. Bull. Env. Pharmacol. Life Sci. 2(11), 139-144.

El-Kaiaty, A.M., Soliman, A.Z.M. & Hassan, M.S.H., 2002. Combine effect of garlic, fenugreek and black seed on some productive and physiological response of laying hens. Egypt. Poultr. Sci. 22, 147-174.

Emery, D., Vohra, P., Ernst, R. & Morrison, S.R., 1984. The effect of cyclic and constant ambient temperatures on feed consumption, egg-production, egg weight, and shell thickness of hens. Poult. Sci. 63(10), 2027-2035.

Eneyew, F. (2006). Effect of fenugreek (*Trigonella foenum graecum*) seeds on voluntary feed intake and nutrients digestibility in crossbred cattle. MSc Thesis, Deemed University NDRI, Karnal.

Fairfull, R. & Chambers, J., 1984. Breeding for feed efficiency: Poultry. Poult. Can. J. Anim. Sci. 64, 513-527.

Farkhoy, M., Modirsane, M., Ghavidel, O., Sadegh, M. & Jafarneyad, S., 2012. Evaluation of protein concentration and limiting amino acids including lysine and Met + Cys in pre-starter diet on performance of broiler. Vet. Med. Inter. 2012, 1-8.

Hassan, H.A. & Ragab, M.S., 2001. The productive performance and egg quality of laying hens under the effect of dietary fenugreek seeds (ungerminated and germinated) and different dietary protein levels. Egypt. Poult. Sci. 1-14.

Hernández, F., Madrid, J., García, V., Orengo, J. & Megías, M.D., 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poult. Sci. 83(2), 169-174.

Lacin, E., Yildiz, A., Esenbuga, N. & Macit, M. 2008. Effects of differences in the initial body weight of groups on laying performance and egg quality parameters of Lohmann laying hens. Czech J. Anim. Sci. 53(11), 466-471.

Leeson, S., Caston, L & Summers, J.D. 1996. Broiler response to diet energy. Poult. Sci. 75, 529-535.

Luiking, Y.C. & Deutz, N.E.P., 2007. Biomarkers of arginine and lysine excess. J. Nutr. 137, 1662S-1668S.

Mahmood, S., Rehman, A., Yousaf, M., Akhtar, P., Abbas, G., Hayat, K., Mahmood, A. & Shahzad, M.K., 2015. Comparative efficacy of different herbal plant's leaf extract on haematology, intestinal histomorphology and nutrient digestibility in broilers. Adv. Zool. Bot. 3(2), 11-16.

Mahmoud, A., Elaroussi, L.R., Forte, S.L. & Harold, V.B., 1994. Calcium homeostasis in the laying hen. Age and dietary calcium effects. Poult. Sci. 73, 1581-1589.

Mamoun, T., Mukhtar, M.A. & Tabidi, M.H., 2014. Effect of fenugreek seed powder on the performance, carcass characteristics and some blood serum attributes. Adv. Res. Agric. Vet. Sci. 1(1), 6-11.

Mashaly, M.M., Hendricks, G.L., Kalama, M., Gehad, E., Abbas, O. & Patterson, P.H., 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poult. Sci. 83(6), 889-894.

Mazur, W.M., Duke, J.A., Wähälä, K., Rasku, S. & Adlercreutz, H., 1998. Isoflavonoids and lignans in legumes: Nutritional and health aspects in humans. J. Nutr. Biochem. 9(4), 193-200.

Moustafa, E.-K., 2006. Effect of using commercial and natural growth promoters on the performance of commercial laying hens. Egypt. Poultr. Sci. 26, 941-965.

Muiruri, H.K. & Harrison, P.C., 1991. Effect of roost temperature on performance of chickens in hot ambient environments. Poult. Sci. 70(11), 2253-2258.

Murray, I.L.K., Granner, D.K., Mayes, P.A. & Rodwell, V.W., 1991. Text book of Harper's biochemistry, 22nd ed. Connecticut/Los Altos, California.

National Research Council US., 1984., 1984. Nutrient requirements of poultry (No. 1). National Academies, Subcommittee on Poultry Nutrition.

Novak, C.L. & Scheideler, S., 1998. The combined effects of lysine and TSAA in two strains of laying hens. J. Poultr. Sci. 77, 102.

Petrolli, T.G., Albino, L.F.T., Rostagno, H.S., Gomes, P.C., Tavernari, F.C. & Balbino, E.M., 2012. Revista Brasileira de Zootecnia plant extracts in diets for broilers. Rev. Bras. Zootec. 41(7), 1683-1690.

Prochaska, J.F., Carey, J.B. & Shafer, D.J., 1996. The effect of L-lysine intake on egg component yield and composition in laying hens. Poult. Sci. 75(10), 1268-1277.

Rama Rao, S.V., Ravindran, V., Srilatha, T., Panda, A.K. & Raju, M.V.L.N., 2011. Effect of dietary concentrations of energy, crude protein, lysine, and methionine on the performance of White Leghorn layers in the tropics. J. Appl. Poult. Res. 20(4), 528-541.

Safaa, H. 2007. Effects of dietary garlic on cholesterol metabolism in laying hens. Egypt. Poult. Sci. 27(IV), 1207-1221.

Samanya, M. & Yamauchi, K., 2002. Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis var. natto*. Comp. Biochem. Physiol. 133, 95-104.

Scott, T.A. & Balnave, D., 1988. Influence of dietary energy, nutrient density and environmental temperature on pullet performance in early lay. Br. Poult. Sci. 29(1), 155-165.

Tollba, A., Abd El-Galyl, M. & Abd El- Samad, M., 2005. The effect of using some herbal additives on physiological and productive performance of two Egyptian chicken strains during winter and summer seasons. Egypt. Poultr. Sci. 25, 107-123.

Whitehead, C.C., 1998. Vitamin E can alleviate the depressed egg production of heat-stressed laying hens. Br. Poult. Sci. 39, 44-46.

Wolfenson, D., Frei, Y.F., Snapir, N. & Berman, A., 1979. Effect of diurnal or nocturnal heat stress on egg formation. Br. Poult. Sci. 20(2), 167-174.

Chapter 4: Egg quality and shelf life

Abstract

The effect of Nutrifen® and Nutrifen Plus® on the egg quality and egg shelf-life for eggs obtained from Hy-Line hens aged between 32 weeks were determined. The egg quality parameters included both external and internal quality parameters. Five treatments were used in the study: a basal diet (control) with a filler and four treatment diets comprising a basal diet substituted with different inclusion levels of Nutrifen® [0.1% (N1) and 0.2% (N2)] and Nutrifen Plus® [0.1% (N+1) and 0.2%(N+2)]. Eggs were collected daily for a duration of 30 days. A total of six hens were selected from each experimental group (6 x 5) that produced an egg every day for 30 days. The egg shelf life of their eggs was evaluated after storing these eggs for three months. Egg quality analysis was performed on the eggs from day 1 to day 15 of the selected six hens in each group within the first month (between day 28 and day 30) of storage. For the second 15 days, eggs (day 16 – 30) were stored for three months (90 day) and thereafter egg quality analysis was performed. The results recorded showed no significant difference between treatments for all the respective egg quality parameters in the first analysis at one month (30 days), except for colour L*. Nutrifen® included at 0.2% showed significant increase in the colour L* (whiteness) value of the egg yolk compared to the control and other treatments. No significant differences were observed among the other treatments regarding the L* colour values. After storing eggs for a period of 90 days, no significant differences (positive or negative) were observed for any of the egg quality parameters except for the L* (whiteness) and b* (yellowness) colour ordinates of the yolk. In addition, the colour fan value had a high tendency to differ among treatments. The L* colour value for treatment Nutrifen® 0.2% was significantly higher than all other treatments and the control, resulting in a whiter egg yolk of these hens. On the other hand, treatment Nutrifen® 0.1% showed a significantly higher b* colour value, which indicates a more yellow colour compared to the control and treatments Nutrifen® 0.2% and Nutrifen Plus® 0.2%. Treatment Nutrifen Plus® 0.1% did not differ significantly from treatment Nutrifen® 0.1% for the b* colour value, however Nutrifen Plus® 0.1% had a higher b* value compared to treatment Nutrifen Plus® 0.2%. In addition, the control treatment had a significantly lower colour fan value than all other treatments, indicating a lighter egg yolk colour of the control group, with no other differences observed among the treatments. In the shelf life study, the comparison between the first egg analysis (30 days) and the second egg analysis (90 days), significant differences (*P*

≤0.05) were observed for the egg weight, yolk weight, albumen weight, yolk height, albumen height, yolk colour L* and b* and the colour fan value between treatments.The egg weight, albumen weight, yolk height and albumen height were significantly decreased after a storage period of three months. On the other hand, the yolk weight significantly increased during storage. Lastly, it was found that the yolk from all treatments were whiter (increased colour L* value) during storage. Only the control, Nutrifen® 0.2% and Nutrifen Plus® 0.2% had a lower b* colour value after storage, which indicates a decrease in the yellowness of the egg.

Keywords: egg quality; egg shelf-life; egg yolk colour

4.1 Introduction

Eggs are a highly nutritious food for humans and are one of the only foods from animal origin that can be stored for long periods (several weeks) in their natural condition, without deterioration to the point where it is unfit for human consumption. The shelf life of eggs is due to their ability to function as a storage unit to protect their content (Grashorn *et al.*, 2016). The layer hen industry has identified egg weight, egg shape, shell thickness, breaking strength, specific gravity, air cell size, and the height and weight of the albumen and yolk as the most important egg quality characteristics (Samli *et al.*, 2005). Therefore, when egg quality is determined, these characteristics are assessed. It is also important to understand the factors that could influence egg quality, especially when the industry's goal is to produce high quality eggs.

Egg quality can be affected by various factors such as nutrition of the hen, hen age, stress and storage. A balanced diet is critical in optimising both production and egg quality parameters. When a balanced diet is supplied, the addition of feed additives can significantly improve and help maintain such parameters. Natural feed additives are being used and investigated more and more as a replacement for synthetic additives. Fenugreek is a natural feed additive that has shown the potential to improve animal production (Abdouli *et al.*, 2014). The high level of bioactivity of fenugreek is likely the cause of numerous positive responses of fenugreek on animal production (Blank *et al.* 1997). Generally, fenugreek is included at low concentrations in poultry diets to serve as a substitute for antibiotics, as it has proven to exert similar effects to antibiotics (Alloui *et al.*, 2012). These effects include stimulating appetite (Hossain *et al*., 2015) and intake (Gad *et al*., 2006), improved weight gain (Lee *et al*., 2007), improved immune status (Hossain *et al.*, 2015) through improved gut health (Petrolli *et al.*, 2012), improved egg weight and quality (Hassan & Ragab, 2001 and Abaza, 2007) and improved reproduction (Awadein *et al.*, 2010; Alobaidy, 2012). In addition, fenugreek is a plant that has been used as a spice in food for many years

(Meghwal & Goswami, 2012). Therefore, fenugreek may play a role in feed intake and its characteristic flavour may also influence the sensory characteristics of an egg. Egg yolk colour and the taste of eggs are important attributes to the consumer; therefore, evaluating the effect of fenugreek on these sensory qualities is important for layer hen farming (Abdouli *et al*., 2014).

The size of the egg is largely affected by the age of the hen. Older hens lay larger eggs with a thinner egg shell, while younger hens lay smaller eggs with thicker shells. This has a significant impact on the quality of the eggs (Akyurek & Okur, 2009). The thickness of the shell and the resistance of the shell to breakage also depends on the supply of dietary calcium, phosphorus and vitamin D_3 (Grashorn *et al.*, 2016). The age of the hens also affects the internal egg quality of freshly laid eggs, as albumen quality rapidly declines in older hens (Samli *et al.*, 2005). Albumen quality is also greatly influenced by genetic factors (Johnson & Merritt, 1955). The albumen quality largely represents the freshness of an egg and is also important for the industry, given that the albumen and the yolk have separate markets (Ahn *et al.*, 1997).

Excluding the hen's physiology and its nutrition, the quality of eggs is also highly affected by environmental conditions such as the storage time, temperature, humidity and the presence of CO_2 (Samli *et al.*, 2005). Several researchers have conducted studies on the effect of storage time and temperature as well as their interaction on the egg quality. Long-term storage, high temperatures and high humidity have been reported to negatively affect the quality characteristics of eggs (Silversides & Scott, 2001; Samli *et al.*, 2005; Yilmaz & Bozkurt, 2009; Akter *et al.*, 2014). Therefore, proper management of these factors during storage are essential for successful storage with minimum egg quality deterioration. During storage, water is lost from the egg through evaporation. This loss of water needs to be prevented in order to maintain egg quality during storage (Walsh *et al.*, 1995). This, together with the flattening of the egg yolk due to the vitelline membrane becoming increasingly weaker, negatively affects the internal egg quality (Fromm & Matrone, 1962). The weakening of the vitelline membrane may be caused by the movement of water due to osmotic pressure from the albumen to the yolk (Akter *et al.*, 2014).

All the aspects discussed above can influence egg quality and hence, the shelf life of an egg. Since nutrition can influence egg quality, it is reasonable to evaluate products (i.e., natural feed additives) that may improve the egg quality through nutrition as egg quality is also directly related to the egg shelf life. Therefore, the aim of the research presented in this chapter was to determine the effect of Nutrifen® and Nutrifen Plus® on the egg quality and egg shelf life of eggs produced by Hy-Line hens.

4.2 Materials and methods

The materials and methods to the point of egg collection are described in detail in Chapter 3. Ethical clearance was obtained from the ethical committee of Stellenbosch University (SU-ACUD16-000099). The practical techniques and practices of this trial were in accordance with the regulations of the University of Stellenbosch's ethical committee.

4.2.1 Egg quality sampling, storage and measurements

Eggs were collected daily at approximately mid-day (12:00). Each egg was marked with the hen numbers and date using a pencil. All the collected eggs were then placed in egg boxes marked with dates, hen numbers and treatment for identification. The eggs were than stored in a laboratory room with temperatures ranging between 15 ºC to 17 ºC and was consistent throughout the storage time. The hen number and eggs laid were monitored and recorded daily. Hens who laid an egg every day for the duration of the trial were identified and used for the egg quality analysis. Hens that laid an egg every day for 30 days could only be identified after 30 days. Egg quality tests were performed on the eggs from six hens per treatment that laid an egg every day for 30 days. The first 15 eggs (day 1 to 15) from each of six birds per treatment were analysed when the eggs were exactly 30 days of age after collection. Therefore the eggs laid on day one of collection was analysed when 30 day old and those laid on day two were analysed the next day when they were 30 days old and so on. The second 15 eggs (days 16 to 30) were stored for 3 months (90 days) and analysed on exactly 90 days of age. Therefore it took 15 days to analyse the batch at 30 days (waited until each egg was exactly 30 days old) and the same for 90 days analysis. No egg was analysed before or after 30 and 90 days age. The parameters used for egg quality analysis are presented in Table 4.1.

All individual eggs that underwent analysis were analysed for both internal and external egg quality characteristics. The process of egg analysis was as follows: The egg was weighed using a laboratory scale (Mettler PC 4400 scale), after which it was candled and examined for external shell characteristics (i.e. bumps and cracks). A digital calliper (Mitutoyo 500-196-20, 150mm, 0.02 accuracy) was used to measure egg height and egg diameter in millimetres (mm). The egg was then broken onto a level glass with a white surface underneath. The wet shell was weighed, and the shell thickness determined (using the digital calliper) by taking measurements at three different places on the egg shell and then taking an average of the three measurements. The size of the air sac was then visually determined, and the height of the egg yolk and albumen (thick and

thin) was measured using a manual tripod micrometer. The spread of the thick and thin white was determined based on how far and wide the albumen has spread from the yolk when broken. The Haugh Unit (HU) represents the visual appearance of an egg once broken out of the shell onto a flat surface. It is measured by the ratio between the whole egg weight and the height of the inner thick albumen. The visual appearance of both the external and internal egg were considered relevant in predicting egg quality (Eisen *et al.*, 1962).

The yolk was then separated from the albumen and weighed. The weight of the albumen was determined by subtracting the weight of the shell and the yolk weight from the weight of the whole egg. The yolk colour was measured with both a colour fan and a digital colour spectrophotometer according to the Hunter L*, a* and b* scale. The spectrophotometer lenses were placed as close as possible and gently on top of the egg yolk for the reading to be taken. A sketch of the Hunters colour scale is shown in Figure 4.1. The scale is in cube form, with the L* axis running from a maximum of 100 (white) at the top and a minimum of 0 (black) at the bottom. The a* and b* axes are not limited by a numerical value; however, a negative or positive value indicates a different colour. The positive and negative a* value indicate the colours red and green, respectively. Positive b indicates the colour yellow and negative b* the colour blue.

Table 4.1 Egg quality parameters

Whole egg quality	Egg shell quality	Egg yolk quality	Egg white quality
[1]Egg weight (g)	[1]Shell thickness (mm)	[1]Yolk weight (g)	[1]Albumin weight (g)
[1]Egg height (mm)	[1]Shell weight (g)	[1]Colour L[c]	[2]Colour (clear/cloudy)
[1]Egg diameter (mm)	[2]Form (l/es/o/d)[a]	[1]Colour a[c]	[2]Blood spots
	[2]Banded	[1]Colour b[c]	[2]Meat spots
	[2]Rough	[2]Colour fan	[1]Thick white height (mm)
	[2]Bumps	[2]Blood spots	[2]Thick white spread (L/M/H)[d]
	[2]Uneven	[2]Meat spots	[1]Thin white height (mm)
	[2]Pigment	[2]Membrane strength	[2]Thin white spread (L/M/H)[d]
	[2]Soft shell	[1]Height (mm)	[2]Chalazae
	[2]Shell-less	[2]Mottled	[2]Membrane strength
	[2]Cracked	[2]Discolouring of yolk	[2]Air sack (s/m/l)[e]
	[2]Star crack		
	[2]Hairline crack		
	[2]Broken		
	[2]Pinhole		
	[2]White		
	[2]Dirty (aa/a/b/d)[b]		

[a] l = long; es = egg shaped; o = oval; d = deformed
[b] aa = clean; a = slightly dirty; b = moderately dirty; d = dirty
[c] Colour L = lightness; Colour a = red/green; Colour b = yellow/blue
[d] L = low grade spread; M = medium grade spread; H = high grade spread
[e] s = small; m = medium, l = large
[1] Numerical data
[2] Categorical data

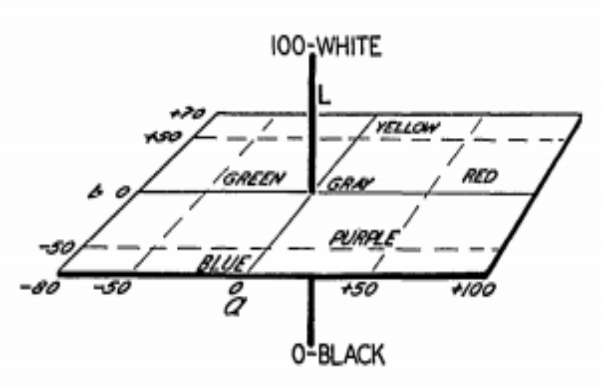

Figure 4.1 Visual representation of the Hunter colour scale with dimensions L, a and b (Hunter, 1958)

4.3 Statistical analysis

The Hypotheses tested:

H_0: There is no relationship between the egg quality parameters and the treatment diets at an egg age of 30 days

H_a: There is evidence to suggest that there is a relationship between the egg quality parameters and the treatment diets at an egg age of 30 days old

H_1: There is no relationship between the egg quality parameters and the treatment diets at an egg age of 90 days

H_{a1}: There is evidence to suggest that there is a relationship between the egg quality parameters and the treatment diets at an egg age of 90 days old

H_2: There is no relationship between the egg quality parameters, the treatment diets and the shelf life of eggs

H_{a2}: There is evidence to suggest that there is a relationship between the egg quality parameters, the treatment diets and shelf life of eggs

The experiment was based on a completely randomised design. The data were analysed using a mixed model analysis of variance (ANOVA), with hen number being a repeated factor and all other factors being fixed effects, using Statistica 64 (Version 13.2, 2017). The other factors included egg quality parameters, treatments, time (months) in storage and the interaction between time and treatment. The significant differences between means were tested using Fisher's least

significant difference (LSD) tests. A probability value of $P \leq 0.05$ (5% significance level) indicated that the difference between treatments were statistically significant.

4.4 Results and Discussion

4.4.1 Month one (30 days)

The results for the egg quality parameters measured within one month are represented in Table 4.2. No significant differences ($P > 0.05$) were found between the treatment diets for egg weight, shell weight, yolk weight, albumen weight, egg height, egg diameter, shell thickness, and the thick and thin albumen height.

The effect of Nutrifen® and Nutrifen Plus® at 0.1 and 0.2% dietary inclusion levels has proven to be insufficient in affecting egg weight in this study. Various researchers (Awadein *et al.*, 2010; Abdouli *et al.*, 2014; Panaite *et al.*, 2015; Omri *et al.*, 2017) who tested the effect of dietary fenugreek at different inclusion levels on egg weight observed similar results. Awadein *et al.* (2010) studied the inclusion of fenugreek at 0.1% and 0.5% on 16-24 weeks of age on open floor pens and found no difference between the two treatment diets and the control for egg weight. Similarly, Panaite *et al.* (2015) studied the inclusion of fenugreek in a layer diet at 1% and 2% on 48-week-old birds kept in cages with three birds per cage, and found no significant improvement of egg weight compared to a control diet. Although one would think that the differences in inclusion levels may affect the results, both Awadein *et al.* (2010) and Panaite *et al.* (2015) tested similar or higher inclusion levels compared to the present study and found no improvement in egg weight. It is also evident that the age of the hens and the housing of the different studies were different. Therefore, the effect of these elements was not significant enough to show different results. Overall, there is more evidence of fenugreek having no effect on egg weight than reported for a positive or negative effect.

Table 4.2 The means ± standard error of egg quality parameters for eggs aged one month from Hy-Line hens fed diets containing different inclusion levels of Nutrifen® and Nutrifen Plus®

	Treatment (diet)					
Parameter	**Control**	**Nutrifen® 0.1**	**Nutrifen® 0.2**	**Nutrifen Plus® 0.1**	**Nutrifen Plus® 0.2**	***P* value**
Egg weight (g)	56.36 ± 1.263	55.48 ± 1.263	57.64 ± 1.263	55.19 ± 1.263	56.52 ± 1.263	0.68
Egg height (mm)	56.73 ± 0.489	55.33 ± 0. 489	56.19 ± 0. 489	55.32 ± 0. 489	56.18 ± 0. 489	0.22
Egg diameter (mm)	42.99 ± 0.407	43.24 ± 0.407	43.45 ± 0.407	42.86 ± 0.407	43.15 ± 0.407	0.87
Shell thickness (mm)	0.40 ± 0.004	0.39 ± 0.004	0.40 ± 0.004	0.39 ± 0.004	0.39 ± 0.004	0.30
Shell weight (g)	7.50 ± 0.171	7.20 ± 0.171	7.55 ± 0.171	7.18 ± 0.171	7.49 ± 0.171	0.34
Yolk weight (g)	16.04 ± 0.451	16.43 ± 0.451	16.36 ± 0.451	16.01 ± 0.451	17.21 ± 0.451	0.40
Yolk colour L*	63.00^{b} ± 0.507	63.27^{b} ± 0.509	65.27^{a} ± 0.507	63.32^{b} ± 0.507	63.96^{ab} ± 0.507	0.02
Yolk colour a*	8.30 ± 0.311	7.77 ± 0.311	8.33 ± 0.311	8.35 ± 0.311	8.43 ± 0.311	0.72
Yolk colour b*	60.68 ± 0.773	61.02 ± 0.776	61.15 ± 0.773	60.84 ± 0.773	60.07 ± 0.773	0.89
Yolk height (mm)	15.42 ± 0.229	15.01 ± 0.229	15.65 ± 0.229	15.51 ± 0.229	15.52 ± 0.229	0.32
Albumen weight (g)	38.48 ± 0.934	37.97 ± 0.935	38.52 ± 0.934	37.55 ± 0.935	39.02 ± 0.934	0.88
Thick albumen height (mm)	2.53 ± 0.099	2.61 ± 0.099	2.65 ± 0.099	2.59 ± 0.099	2.53 ± 0.099	0.87
Thin albumen height (mm)	2.07 ± 0.099	2.23 ± 0.099	2.16 ± 0.099	2.02 ± 0.099	2.09 ± 0.099	0.60
Bands (%)	0.22 ± 0.090	0.19 ± 0.090	0.42 ± 0.090	0.14 ± 0.090	0.27 ± 0.090	0.26
Bumps (%)	0.32 ± 0.090	0.14 ± 0.090	0.30 ± 0.090	0.24 ± 0.090	0.14 ± 0.090	0.44
Pigment (%)	0.62 ± 0.156	0.47 ± 0.156	0.66 ± 0.156	0.77 ± 0.156	0.71 ± 0.156	0.72
Yolk blood spot (%)	0.25 ± 0.072	0.19 ± 0.072	0.36 ± 0.072	0.46 ± 0.072	0.32 ± 0.072	0.14
Yolk membrane (%)	0.98 ± 0.008	1.00 ± 0.008	0.99 ± 0.008	0.99 ± 0.008	0.99 ± 0.008	0.75
Yolk meat spots (%)	0.08 ± 0.024	0.05 ± 0.024	0.07 ± 0.024	0.04 ± 0.024	0.08 ± 0.024	0.63
Mottled yolk (%)	0.08 ± 0.024	0.05 ± 0.024	0.07 ± 0.024	0.04 ± 0.024	0.08 ± 0.024	0.63
Albumen blood spots (%)	0.33 ± 0.089	0.42 ± 0.089	0.40 ± 0.089	0.48 ± 0.089	0.41 ± 0.089	0.85
Albumen meat spots (%)	0.14 ± 0.041	0.18 ± 0.041	0.13 ± 0.041	0.16 ± 0.041	0.17 ± 0.041	0.89
Colour fan	7.96 ± 0.021	7.94 ± 0.021	7.91 ± 0.021	7.94 ± 0.021	7.95 ± 0.021	0.06
Thick white spread	0.33 ± 0.105	0.33 ± 0.105	0.62 ± 0.105	0.46 ± 0.105	0.30 ± 0.105	0.19
Thin white spread	0.30 ± 0.093	0.32 ± 0.093	0.57 ± 0.093	0.39 ± 0.093	0.26 ± 0.093	0.18
Air sac	1.10 ± 0.071	0.91 ± 0.071	0.95 ± 0.071	0.90 ± 0.071	1.13 ± 0.071	0.08

(^{a,b}) Means with different subscripts in the same row differ significantly ($P \leq 0.05$).

(%) The percentage of eggs that were observed or found to have these traits within a treatment group.

In one study by Moustafa (2006), a significant improvement in the egg weight of fenugreek fed hens compared to a control diet was found. At an inclusion level of 0.05%, Moustafa (2006) found that the egg weight of 40-59-week-old hens were significantly heavier compared to a control diet. The differences observed may be due to the various inclusion levels, hen age, housing density and temperature. The hens from this study and that of Moustafa (2006) were of different ages. During the present study, hens were at peak production and it is likely that the unaffected egg weight may be attributed to this. There is a 10-15 week difference in age between hens from this study and that of Moustafa (2006). An increase in hen age has proven to significantly increase the egg weight (Silversides & Scott, 2001; Tůmová & Goust, 2012). An increase in hen age also increases the bird's nutrient requirements, which can result in noticeable differences in egg weight (Tůmová & Goust, 2012). Panaite *et al.* (2015) however, studied the effect of fenugreek seed at two inclusion levels (1% and 2%) on 48-week-old Lohmann Brown layers and still concluded no improvement in egg weight despite a similar hen age to those in Moustafa's (2006) study. Therefore, hen age can be removed as a possible reason for the increase in egg weight. In addition, the stocking density and temperature may also affect the hen's production. The birds from this trial were not exposed to any stress in terms of over-stocking and extreme temperatures during the trial. High stocking densities and extreme high and low temperatures negatively impact egg weight (Tůmová & Goust, 2012).

Considering this information gathered, there is very little evidence of a positive effect of fenugreek on egg weight. Older hens may respond better to the addition of fenugreek as a natural feed additive due to their increased nutrient requirement and physiological stress experienced. In addition, a variety of concentrations should be tested on both young and older birds under similar environmental conditions, as these factors may affect the results.

The shell weight and shell thickness were unaffected during this study (P >0.05), similar to the study by Omri *et al.* (2017) who studied the effect of a standard diet supplemented with 2% fenugreek. The inclusion levels of this study and that of Omri *et al.* (2017) differ greatly and therefore, the inclusion levels may not be a plausible reason for the unaffected response. Omri *et al.* (2017) tested birds at 27 weeks of age that were housed in individual cages as in the present study. The animals only received 100g of feed per day, while in this study birds were fed *ad libitum*. The birds in this study therefore may have ingested more fenugreek while Omri *et al.* (2017) birds were restricted. This however did not change the outcome of the results of the shell weight and shell thickness.

However, Abdouli *et al.* (2014) and Moustafa (2006) found significant improvement of the shell weight and shell thickness of eggs from hens fed fenugreek diets. Surprisingly, the inclusion levels of

the studies that found significant improvements in shell weight and thickness had similar inclusions to the studies that found insignificant results. The age (39-41 weeks) of the hens from the study of Abdouli *et al.* (2014) and Moustafa (2006) (40-59 weeks) was slightly higher than the hens of the present study. The calcium requirement increases with increasing hen age and has a direct effect on egg shell quality (Tůmová & Goust, 2012). Fenugreek may have positively contributed to calcium availability and absorption during this period of stress (older birds) resulting in improved egg shell quality for these two studies. Hence, the difference in age may have produced the difference in results and it could be postulated that hens may perform better on Nutrifen® supplemented diets when they are older.

The yolk weight, albumen weight and the height of the yolk and albumen has proven to be unaffected by the treatments of this study. The yolk weight and albumen weight are directly related to the overall egg weight. Omri *et al.* (2017) and Ahmad *et al.* (2016) also found no significant improvement of the egg yolk and albumen weight with supplementation of fenugreek. Differences in hen age and inclusion levels between these studies still produced similar results. In contradiction to these results, Abdouli *et al.* (2014) and El-Kaiaty *et al.* (2010) found a significant improvement of yolk and albumen weight. Panaite *et al.* (2015) also found an improvement in yolk weight and reduction in albumen height. The differences in results may once again be due to the inclusion levels or the age of the hens for the respective trails. The hens from the study of Panaite *et al.* (2015) were 49 weeks old, which is higher than the present study and that of Omri *et al.* (2017). Older hens are known to lay larger and heavier eggs. The total egg weight is directly affected by an increase in yolk weight or albumen weight or both. There are reports of egg yolk increasing while egg albumen decreases, vice versa and an increase in both with the increase in egg weight (Suk & Park, 2001). The increase in the amount of albumen in the egg is due to a decrease in albumen solids (Travel & Nys, 2011). Genetic variation and the storage time before analysis also differed between the studies. This may significantly influence the results especially with regards to yolk and albumen quality, as they deteriorate with increased storage time (Samli *et al.*, 2005). The studies discussed above evaluated the egg quality parameters much earlier than the present study (between one day and two weeks). It is evident that the internal egg quality deteriorates with every day in storage (Samli *et al.*, 2005).

The percentage bands, bumps, pigment, yolk blood and meat spots, yolk membrane, mottled yolks and albumen blood and meat spots was unaffected by the treatment diets (P >0.05) for the respective weeks and overall period of the trial. The colour fan score, thick and thin albumen spread, and the air sac size was also unaffected (P >0.05) by the treatments. Blood and meat spots are usually influenced by the age of the hen and the strain of the birds (Ahmadi & Rahimi, 2011). There is very little to no statistical evidence to support the results found in this study regarding egg bands, bumps and pigments.

However, the qualities being unaffected are positive results as it implies that the fenugreek products, Nutrifen® and Nutrifen Plus®, do not negatively influence the quality of egg shells.

Although colour a* and b* of the yolk show no significant differences (P >0.05) between treatments, the colour L* (lightness) value was significantly (P ≤0.05) affected by the treatment diets. The N2 treatment had a significantly higher colour L* value compared to that of the control, N1 and N+1 (Table 4.2) while N2 did not significantly differ (P >0.05) from N+2. No significant differences were observed between the other treatments and the control. It is possible that the difference in concentration may have led to the differences observed between N1 and N2. The differences that was observed between N2 and N+1 may be due to the difference in ingredients of the two products, Nutrifen® and Nutrifen Plus®. The greater concentration of Nutrifen Plus® (N+2) yield results similar to that of N2, therefore indicating that the higher concentrations may respond in terms of the colour L*. The response in this case with a higher concentration of Nutrifen® (N2) is negative as consumers have expressed a greater interest in a yellow to dark yellow egg (Bovšková *et al.*, 2014). Evaluating different concentrations of these products on egg yolk colour may yield better results in terms of its positive or negative effect on yolk colour (i.e., a*, b* and L*).

In a study by Abdouli *et al.* (2014) on the effect of fenugreek at 0, 2, 4 and 6 g/hen/day on egg colour and sensory qualities in Lohmann white layers (52 weeks of age), the egg colour was evaluated using a yolk colour fan scale (RYCF) and the colour L* and b* using a Konica Minolta Chroma Meter CR-410. A significant increase was observed in colour L* by the addition of fenugreek seeds at all concentrations. No significant differences were observed between the control and all treatments for egg yolk colour fan score (RYCF) and colour b* (yellowness). These results are in accordance with the present study, where the inclusion of fenugreek in the diet only significantly increased the colour L* value of the yolk which causes an increase in whiteness of the egg. This may result in a slightly lighter appearance of the egg yolk, which is not desired as it has been shown through surveys that consumers are more interested in deep yellow egg yolk compared to a light appearance. Consumers regard egg yolk as one of the main egg quality parameters upon which they judge an egg (Beardsworth & Hernandez, 2004). Abdouli *et al.* (2014) also reported that at higher inclusion levels (>2 g), fenugreek seemed to improve egg yolk redness. This study and the study by Abdouli *et al.* (2014) showed no change in the yellowness (a value) of the egg yolk. The reason for this may be due to yellow maize being supplied in equal amounts for all diets, including the control. The inclusion levels of the Nutrifen® and Nutrifen Plus® in the present study may have been insufficient to supply pigment and therefore did not affect the yellow colour of the egg yolk.

4.4.2 Month three (90 days)

There is limited research on the effect of fenugreek, included in the diets of layer hens, on the storage life of eggs. However, it is evident that any factor that improves egg quality could improve the long-term storage of layer eggs under the right conditions (Adamic *et al.*, 2002). The results for the comparison between the one-month and the three-month egg quality analysis are shown in Table 4.3. At three months, no significant differences (P >0.05) were observed between treatments for most of the egg quality parameters evaluated (Table 4.3) except for the L* and b* colour values of the yolk.

The results of the L* colour value between treatments were similar to those observed on month one. Treatment N2 had a significantly higher L* colour value compared to all three other treatments and the control. This indicates that after three months of storage, the N2 treatment had a significantly lighter (whiter) egg yolk colour than the eggs from the other treatments. In the first analysis, this was already evident, which indicates that this may not have been due to a storage effect. A whiter egg yolk is not desirable amongst consumers (Bovšková *et al.*, 2014). The inclusion of Nutrifen® at 2% may have been too concentrated, resulting in the increase whiteness of the egg yolk. The yolk colour is determined by dietary xanthophyll pigment of the feed (Karunajeewa *et al.*, 1984). Marusich and Bauernfeind, (1970) found that an increase in natural xanthophyll pigment supplied to a hen in feed decreases the amount deposited into the yolk. This may be why the higher concentration of Nutrifen® increased the whiteness factor of the egg yolk. The N+2 diet was similar to the control, N1 and N+1 treatments, which may indicate that a component of the Nutrifen Plus® additive may have improved the deposition of xanthophyll into the yolk at a higher concentration.

In addition, the yolk's b* colour, which is an indication of the yellowness of the egg yolk and had no effect at month one of analysis, had a significant effect between treatments at month three. Treatment N1 had a significantly (P ≤0.05) higher b* colour values compared to that of the control and treatments N2 and N+2. In addition, Treatment N+1 also has a significantly higher (P ≤0.05) b* colour value than treatment N+2. This indicated that N1 and N+1 had a greater ability to maintain egg yolk yellowness during storage. The lower inclusion levels of treatment N1 and N2 may have increased its ability to deposit its xanthophyll pigment content in the yolk compared to those of higher concentrations, therefore resulting in a more yellow yolk colour in these treatments eggs (Marusich & Bauernfeind, 1970).

The colour fan value (P = 0.06) showed a tendency to differ between treatments. The colour fan values from the control diet was considerably lower than the Nutrifen® and Nutrifen Plus® treatment diets. Given that no significant differences were observed for the colour fan values between treatments

at month one, the differences observed at month three could indicate that Nutrifen® and Nutrifen Plus® have the ability to preserve the colour of the yolk during longer storage periods.

4.4.4 Shelf life (Changes from month one to month three)

The effect of long-term storage on egg quality of hens fed Nutrifen® and Nutrifen Plus® diets was evaluated to determine egg quality parameters after a three-month storage period. There were no significant (P >0.05) differences found between month one and month three between all treatments for egg diameter, shell weight, yolk a* colour, albumen weight and the thick and thin albumen heights, the percentage banded, bumps, pigment, yolk blood spots, yolk membrane, yolk meat spots, mottled yolk, albumen blood spots and albumen meat spots. These results were expected as these factors are a function of each individual hen.

Significant differences (P ≤0.05) were however observed between some or all treatments for egg weight, egg height, shell thickness, yolk weight, yolk L* and b* colour ordinates, yolk height, albumen weight, thick and thin albumen height and the colour fan values after long-term storage.

The egg weight of the control, N1 and N2 decreased significantly (P ≤0.05) during the three-month storage period, while that of N+1 and N+2 decreased, but not significantly over the same period in storage. During storage, an egg loses water and CO_2 through the pores of the egg shell, which causes a reduction in the yolk and albumen weight (Akyurek & Okur, 2009). This directly affects the total egg weight and therefore explains the results observed by this study. The yolk weight was significantly increased (P ≤0.05) for the control, N1 and N2, while the increase in yolk weight for N+1 and N+2 was not significant after storage. Other studies reported an increase in the yolk weight due to increased water movement from albumen to the yolk, which in turn weakens the vitelline membrane (Kirunda & McKee, 2000). The increased water transfer from the albumen to the yolk causes the vitelline membrane to break, resulting in flat and mottled yolks. Oleforuh-Okoleh and Eze (2016) also reported an increase in the yolk weight with increased storage.

Table 4.3 The comparison of the egg quality data from eggs from layer hens aged one month and three months when hens are fed one of five different treatment diets

Month (average)	Treatment (diet) Control	Nutrifen® 0.1	Nutrifen® 0.2	Nutrifen Plus® 0.1	Nutrifen Plus® 0.2	*P* value
Egg weight (g)						
1 Month	56.36 ± 1.316	55.48 ± 1.316	57.64 ± 1.316	55.19 ± 1.316	56.52 ± 1.316	0.68
3 Months	55.07 ± 1.316	54.35 ± 1.316	55.84 ± 1.316	54.78 ± 1.316	56.06 ± 1.316	0.89
P value for shelf life	0.02	0.03	0.00	0.43	0.36	0.26
Egg height (mm)						
1 Month	56.73 ± 0.489	55.33 ± 0. 489	56.19 ± 0. 489	55.32 ± 0. 489	56.18 ± 0. 489	0.16
3 Months	56.76 ± 0.489	55.62 ± 0. 489	56.16 ± 0. 489	55.66 ± 0. 489	56.68 ± 0. 489	0.39
P value for shelf life	0.89	0.18	0.89	0.12	0.03	0.41
Egg diameter (mm)						
1 Month	42.99 ± 0.407	43.24 ± 0.407	43.45 ± 0.407	42.86 ± 0.407	43.15 ± 0.407	0.87
3 Months	42.81 ± 0.407	43.08 ± 0.407	43.41 ± 0.407	43.05 ± 0.407	43.20 ± 0.407	0.87
P value for shelf life	0.42	0.46	0.85	0.39	0.82	0.74
Shell thickness (mm)						
1 Month	0.40 ± 0.004	0.39 ± 0.004	0.40 ± 0.004	0.39 ± 0.004	0.39 ± 0.004	0.30
3 Months	0.38 ± 0.004	0.38 ± 0.004	0.40 ± 0.004	0.39 ± 0.004	0.39 ± 0.004	0.29
P value for shelf life	0.0	0.05	0.55	0.93	0.96	0.09
Shell weight (g)						
1 Month	7.50 ± 0.171	7.20 ± 0.171	7.55 ± 0.171	7.18 ± 0.171	7.49 ± 0.171	0.34
3 Months	7.53 ± 0.171	7.26 ± 0.171	7.63 ± 0.171	7.29 ± 0.171	7.55 ± 0.171	0.49
P value for shelf life	0.68	0.39	0.28	0.15	0.43	0.96
Yolk weight (g)						
1 Month	16.04 ± 0.451	16.43 ± 0.451	16.36 ± 0.451	16.01 ± 0.451	17.21 ± 0.451	0.40
3 Months	16.60 ± 0.451	17.22 ± 0.451	17.06 ± 0.451	16.36 ± 0.451	17.32 ± 0.451	0.49
P value for shelf life	0.03	0.00	0.01	0.17	0.64	0.35
Yolk colour L						
1 Month	63.00^{b} ± 0.507	63.98^{b} ± 0.509	65.27^{a} ± 0.507	63.32^{b} ± 0.507	63.96^{ab} ± 0.507	0.02
3 Months	64.98^{b} ± 0.507	65.17^{b} ± 0.507	67.51^{a} ± 0.507	64.91^{b} ± 0.509	65.59^{b} ± 0.507	0.01
P value for shelf life	0.00	0.00	0.00	0.01	0.01	0.00

(^{a,b}) Means with different subscripts in the same row differ significantly ($P \leq 0.05$).

Table 4.3 Continued

Month (average)	Treatment (diet) Control	Nutrifen® 0.1	Nutrifen® 0.2	Nutrifen Plus® 0.1	Nutrifen Plus® 0.2	*P* value
Yolk colour (a)						
1 Month	8.30 ± 0.311	7.77 ± 0.311	8.33 ± 0.311	8.35 ± 0.311	8.43 ± 0.311	0.72
3 Months	8.48 ± 0.311	8.28 ± 0.311	8.08 ± 0.311	7.79 ± 0.311	8.41 ± 0.311	0.31
P value for shelf life	0.56	0.11	0.42	0.07	0.94	0.16
Yolk colour (b)						
1 Month	60.68 ± 0.773	61.02 ± 0.776	61.15 ± 0.773	60.84 ± 0.773	60.07 ± 0.773	0.89
3 Months	57.59^{bc} ± 0.773	59.56^{a} ± 0.773	57.49^{bc} ± 0.773	59.32^{ab} ± 0.776	56.34^{c} ± 0.773	0.01
P value for shelf life	0.00	0.26	0.00	0.10	0.00	0.02
Yolk height (mm)						
1 Month	15.42 ± 0.229	15.01 ± 0.229	15.65 ± 0.229	15.51 ± 0.229	15.52 ± 0.229	0.32
3 Months	14.39 ± 0.229	14.14 ± 0.229	14.62 ± 0.229	14.75 ± 0.229	14.81 ± 0.229	0.29
P value for shelf life	0.00	0.00	0.00	0.00	0.00	0.00
Albumen weight (g)						
1 Month	38.48 ± 0.934	37.97 ± 0.935	38.52 ± 0.934	37.55 ± 0.935	39.02 ± 0.934	0.88
3 Months	34.41 ± 0.934	33.49 ± 0.935	33.80 ± 0.934	32.52 ± 0.934	33.64 ± 0.934	0.60
P value for shelf life	0.00	0.00	0.00	0.00	0.00	0.00
Thick albumen height (mm)						
1 Month	2.53 ± 0.099	2.61 ± 0.099	2.65 ± 0.099	2.59 ± 0.099	2.53 ± 0.099	0.87
3 Months	2.07 ± 0.099	2.23 ± 0.099	2.16 ± 0.099	2.02 ± 0.099	2.09 ± 0.099	0.60
P value for shelf life	0.00	0.00	0.00	0.00	0.00	0.00
Thin albumen height (mm)						
1 Month	1.63 ± 0.054	1.63 ± 0.054	1.65 ± 0.054	1.66 ± 0.054	1.58 ± 0.054	0.77
3 Months	1.34 ± 0.054	1.34 ± 0.054	1.30 ± 0.054	1.16 ± 0.054	1.30 ± 0.054	0.21
P value for shelf life	0.00	0.00	0.00	0.00	0.00	0.00
Banded (%)						
1 Month	0.22 ± 0.090	0.19 ± 0.090	0.42 ± 0.090	0.14 ± 0.090	0.27 ± 0.090	0.26
3 Months	0.10 ± 0.091	0.14 ± 0.092	0.12 ± 0.092	0.18 ± 0.096	0.30 ± 0.088	0.54
P value for shelf life						0.40

(^{a,b}) Means with different subscripts in the same row differ significantly ($P \leq 0.05$).

(%) The percentage of eggs that were observed or found to have these traits within a treatment group.

Table 4.3 Continued

Month (average)	Treatment (diet) Control	Nutrifen® 0.1	Nutrifen® 0.2	Nutrifen Plus® 0.1	Nutrifen Plus® 0.2	*P* value
Bumps (%)						
1 Month	0.32 ± 0.090	0.14 ± 0.090	0.30 ± 0.090	0.24 ± 0.090	0.14 ± 0.090	0.44
3 Months	0.06 ± 0.065	0.20 ± 0.066	0.25 ± 0.066	0.12 ± 0.069	0.09 ± 0.064	0.23
P value for shelf life						0.29
Pigment (%)						
1 Month	0.62 ± 0.156	0.47 ± 0.156	0.66 ± 0.156	0.77 ± 0.156	0.72 ± 0.156	0.72
3 Months	0.58 ± 0.120	0.55 ± 0.120	0.75 ± 0.120	0.51 ± 0.127	0.71 ± 0.116	0.63
P value for shelf life						0.76
Yolk blood spot (%)						
1 Month	0.25 ± 0.072	0.19 ± 0.072	0.36 ± 0.072	0.46 ± 0.072	0.32 ± 0.072	0.14
3 Months	0.21 ± 0.076	0.07 ± 0.077	0.11 ± 0.077	0.23 ± 0.080	0.30 ± 0.074	0.22
P value for shelf life						0.53
Yolk membrane (%)						
1 Month	0.98 ± 0.008	0.99 ± 0.008	0.99 ± 0.008	0.99 ± 0.008	0.99 ± 0.008	0.75
3 Months	0.85 ± 0.054	0.78 ± 0.055	0.72 ± 0.055	0.78 ± 0.058	0.88 ± 0.054	0.23
P value for shelf life						0.30
Yolk meat spots (%)						
1 Month	0.08 ± 0.024	0.05 ± 0.024	0.07 ± 0.024	0.04 ± 0.024	0.08 ± 0.024	0.63
3 Months	0.03 ± 0.017	0.03 ± 0.018	0.07 ± 0.018	0.06 ± 0.019	0.01 ± 0.018	0.18
P value for shelf life						0.25
Mottled yolk (%)						
1 Month	0.08 ± 0.024	0.05 ± 0.024	0.07 ± 0.024	0.04 ± 0.024	0.08 ± 0.024	0.63
3 Months	0.03 ± 0.017	0.03 ± 0.018	0.07 ± 0.018	0.06 ± 0.018	0.01 ± 0.018	0.18
P value for shelf life						0.26

([a,b]) Means with different subscripts in the same row differ significantly ($P \leq 0.05$).

(%) The percentage of eggs that were observed or found to have these traits within a treatment group.

Table 4.3 Continued

Month (average)	Treatment (diet) Control	Nutrifen® 0.1	Nutrifen® 0.2	Nutrifen Plus® 0.1	Nutrifen Plus® 0.2	*P* value
Albumen blood spots (%)						
1 Month	0.34 ± 0.089	0.42 ± 0.089	0.40 ± 0.089	0.48 ± 0.089	0.41 ± 0.089	0.84
3 Months	0.24 ± 0.069	0.24 ± 0.070	0.27 ± 0.070	0.29 ± 0.074	0.12 ± 0.069	0.49
P value for shelf life						0.79
Albumen meat spots (%)						
1 Month	0.14 ± 0.041	0.18 ± 0.041	0.13 ± 0.041	0.16 ± 0.041	0.17 ± 0.041	0.89
3 Months	0.23 ± 0.074	0.38 ± 0.075	0.22 ± 0.075	0.32 ± 0.078	0.21 ± 0.073	0.45
P value for shelf life						0.85
Colour fan						
1 Month	7.96 ± 0.021	7.94 ± 0.021	7.91 ± 0.021	7.94 ± 0.021	7.95 ± 0.021	0.61
3 Months	7.53^{b} ± 0.036	7.67^{a} ± 0.036	7.64^{a} ± 0.036	7.63^{a} ± 0.036	7.65^{a} ± 0.036	0.06
P value for shelf life						0.02
Thick white spread						
1 Month	0.33 ± 0.105	0.33 ± 0.105	0.62 ± 0.105	0.46 ± 0.105	0.30 ± 0.105	0.19
3 Months	0.22 ± 0.073	0.19 ± 0.074	0.13 ± 0.074	0.22 ± 0.077	0.30 ± 0.075	0.60
P value for shelf life						0.08
Thin white spread						
1 Month	0.30 ± 0.093	0.32 ± 0.093	0.57 ± 0.093	0.39 ± 0.093	0.26 ± 0.093	0.18
3 Months	0.04 ± 0.016	0.04 ± 0.016	0.03 ± 0.016	0.05 ± 0.016	0.00 ± 0.016	0.26
P value for shelf life						0.15
Air sac						
1 Month	1.10 ± 0.071	0.91 ± 0.071	0.95 ± 0.071	0.90 ± 0.071	1.13 ± 0.071	0.08
3 Months	0.97 ± 0.024	0.96 ± 0.024	1.01 ± 0.024	0.99 ± 0.024	1.03 ± 0.024	0.26
P value for shelf life						0.12

(^{a,b}) Means with different subscripts in the same row differ significantly ($P \leq 0.05$).

(%) The percentage of eggs that were observed or found to have these traits within a treatment group.

.

In addition, the albumen weight declined significantly ($P \leq 0.05$) for all treatments and the control during the three-month storage. The decline in albumen weight can be explained by the loss of water and CO_2 from the egg through the shell, causing a rapid increase in the pH of the albumen. The high pH causes the ovomucin and lysozymes in the albumen to dissolve, resulting in the breakdown of albumen, causing it to become watery (Grashorn & Simonovic, 2010). These results indicate a clear difference between Nutrifen® and Nutrifen Plus® in terms of egg weight, albumen and yolk weight loss during storage. The Nutrifen Plus® treatments at both inclusion levels were less affected by storage. This could therefore indicate that Nutrifen Plus® slowed the exchange of water and CO_2, resulting in a reduced egg weight loss. The N1 and N2 treatments had similar responses in terms of the above-mentioned parameters, which means that the inclusion level did not have an effect and could rather be explained by the difference in product ingredients of Nutrifen® and Nutrifen Plus®. Nutrifen Plus® has a more complex composition of ingredients compared to Nutrifen®, and any of their other ingredient may have contributed positively to the effect of Nutrifen Plus® on preserving egg quality during long-term storage.

Samli *et al.* (2005), Oleforuh-Okoleh and Eze (2016), Jin *et al.* (2011) and Eke *et al.* (2013) found similar results with regard to a reduction in egg weight. The eggs for this trial weighed on average between 59 and 66 g, which makes them “large” according to the egg classification of South Africa (Table 2.1). Akter *et al.* (2014) studied the effect of storage and temperature on egg quality of ISA brown layer hens at 44 weeks of age. The eggs were stored at 4ºC and 28-31ºC, and the weight of the eggs decreased over time despite the difference in storage temperature. The egg weight also decreased more with an increase in storage time. In addition, Eke *et al.* (2013) found a significant decrease in egg weight over the four weeks of storage and reported that an increase in the number of shell spores of eggs may be a reason for the reduction in egg weight. In this study, the first analysis was conducted when the eggs were one month old, which may have affected the outcome of the changes observed between the month one and month three analysis because egg quality changes from the day the egg is laid. All the above-mentioned studies did not include fenugreek in the diet, therefore the inclusion Nutrifen Plus® in the diet of the hens for this study might have contributed to the unchanged egg weight observed during storage for N+1 and N+2. Yet, this assumption does not clarify why the control did not differ from the other treatments. The age of the hens is also significantly different between this study and that of Akter *et al.* (2014) and Samli *et al.* (2005), as they used much older hens. This may have had a significant effect on the changes they observed.

In addition, a significant decline ($P \leq 0.05$) was also observed between month one and month three for yolk height and the thick and thin albumen height for all treatments and the control. The yolk height, thick and thin albumen height at month one was significantly higher than that of the eggs at month three for all the treatments. These results were expected, as the longer the storage period

of the eggs, the weaker the yolk membrane becomes, due to an increase in pH of the yolk and albumen as water and CO_2 is lost. This allows the yolk to spread and flatten, resulting in a lower yolk and albumen height (Jones *et al.*, 2002).

There were also significant differences observed in the L* (whiteness) and b* (yellowness) colour values of the egg yolk. The L* colour value of the egg yolk at month three was significantly ($P \leq 0.05$) higher than the eggs at month one for all the treatments. This indicated an increase in the whiteness of all the eggs during storage. The differences are also illustrated in Figure 4.2. During storage, the moisture on the surface of the yolk increases which may cause more light to be reflected, resulting in a higher L* colour value of the yolk. The increase L* colour value is also in accordance with the significant decline ($P \leq 0.05$) in the b* colour value of the yolk for all treatments and the control during the three months storage period (Figure 4.3), which indicates a decline in the yellowness of the egg resulting in a darker appearance (towards the blue end of the colour scale as indicated previously in Figure 4.1). Therefore, none of the treatment diets were effective in reducing the effect of storage on egg yolk colour change. Jin *et al.* (2011) also observed a change in the colour of eggs over time and reported a darker colour in eggs stored for a long period of time. The pH of the yolk increases significantly during storage (Akter *et al.*, 2014). This, together with dehydration due to the loss of water, may result in darker or discoloured egg yolks.

In addition, a significant decline ($P \leq 0.05$) was found in the colour fan value from month one to month three, with month three having a significantly lower colour fan value, irrespective of diet. Figure 4.4 shows the significant differences that exist between all treatments for the respective months for colour fan values. The lower colour fan value at month three indicates a lightening in colour from a darker yellow to a lighter or paler yellow of the eggs during storage. The increase in the L* colour value supports this finding of a lighter egg yolk observed. This may be due to a change in the light reflection of the egg yolk in aged eggs due to internal changes and membrane weakening.

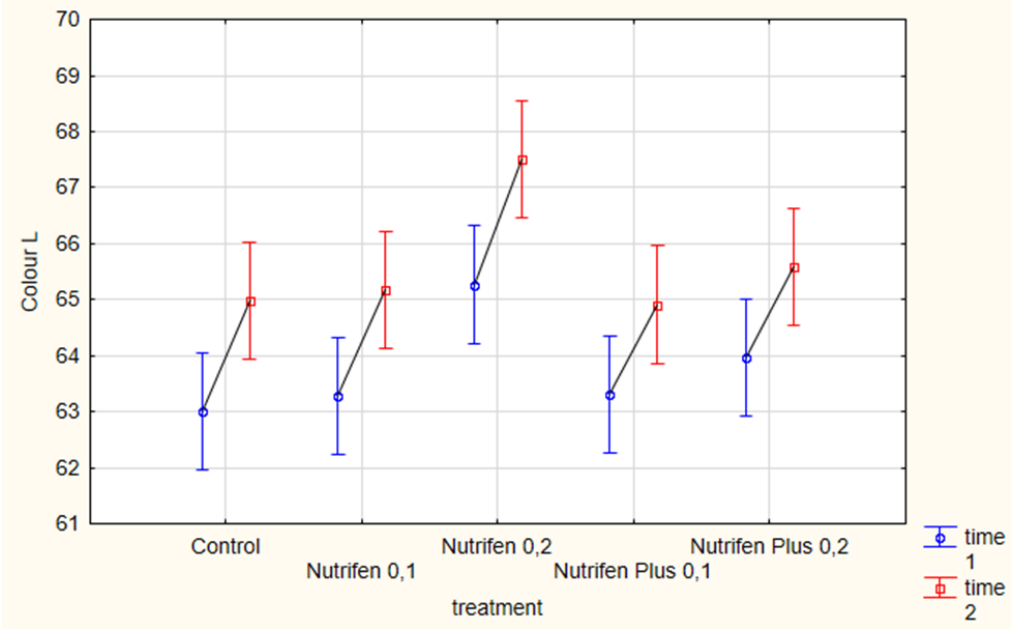

Figure 4.2 The effect of the treatment diets on yolk colour L* value of eggs from layer hens when eggs are aged from time one (30 days) to time two (90 days)

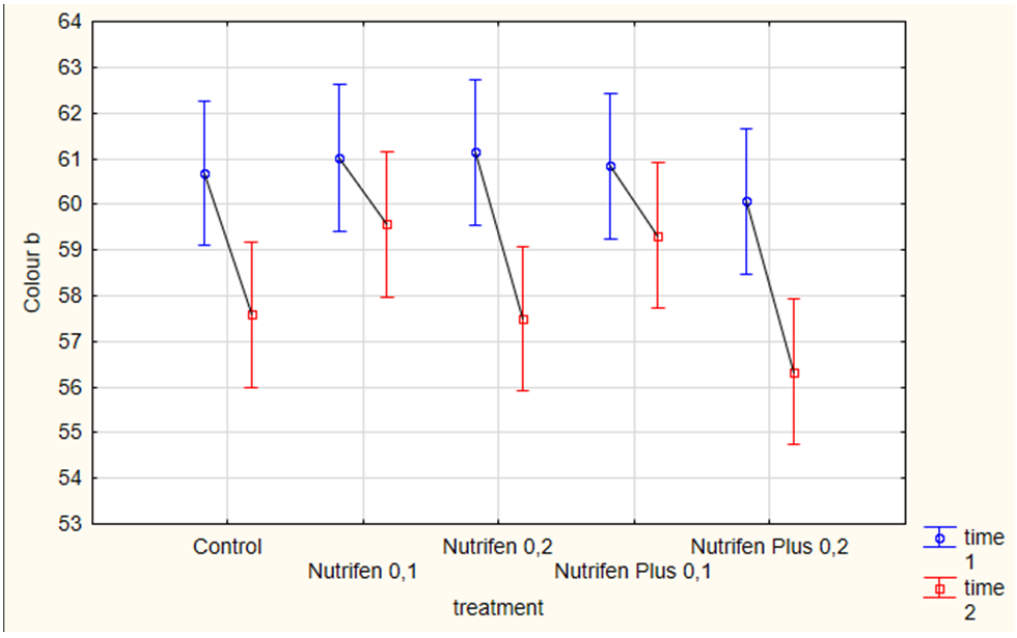

Figure 4.3 The effect of the treatment diets on the yolk colour b* value of eggs from layer hens when eggs are aged from time one (30 days) to time two (90 days)

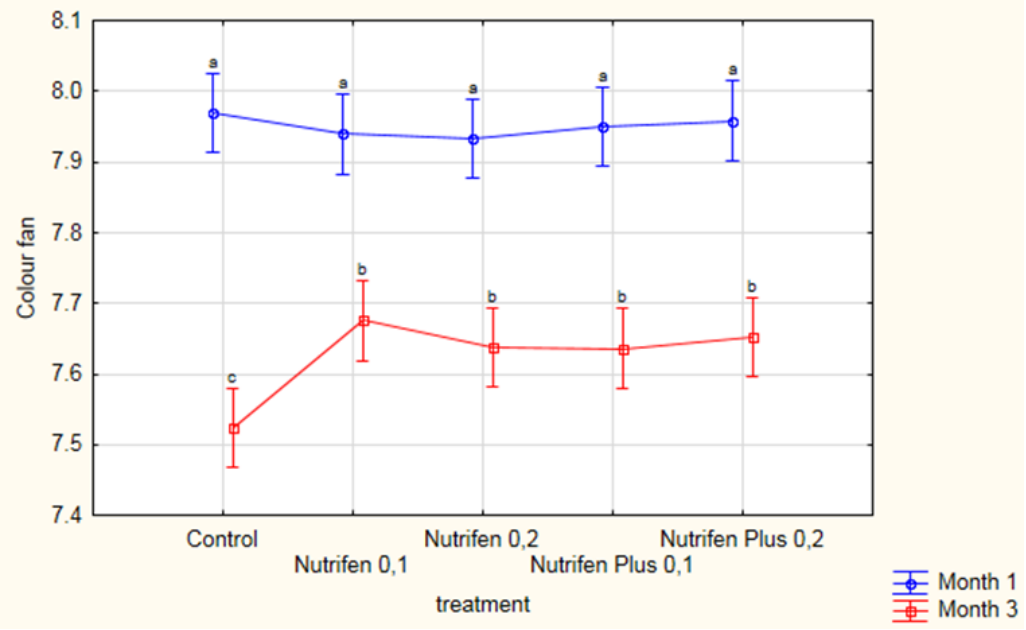

Figure 4.4 The effect of the treatment diets on the yolk colour fan value of eggs from layer hens when eggs are aged one month and three months

Although not significant (P >0.05), the differences between month one and three were evident in the thick white spread at a 10% significance (P ≤0.1) level. All the treatments except N2 showed no differences (P >0.05) between month one and three. N2 shows a significant difference in the thick white spread, with month three being wider spread than month one. The increase in thick white spread with time (as observed for N2) is normal during storage, as reported by various studies (Jin *et al.*, 2011; Eke *et al.*, 2013; Oleforuh-Okoleh & Eze, 2016). This may be due to the degradation of the albumen membrane during storage (Kirunda & McKee, 2000). This observation is also supported by findings from Akter *et al.* (2014), Samli *et al.* (2005) and Grashorn *et al.* (2016), describing a decline in albumen quality during storage. A reduction in albumen quality may be due to an increase in pH caused by the breakdown of the ovomucin-lysozyme complex (Akter *et al.*, 2014). The unaffected thick white spread of the other treatments does not necessarily mean that Nutrifen® and Nutrifen Plus® were able to decrease the effect of albumen degradation, as the control diet was also unaffected. The spread of the egg albumen was done visually, which may have affected the outcome of the results.

This study found no significant differences in the size of the air sac between treatments for the respective months. These results were not expected as the air sac gradually increases with increase storage time do to the loss of CO_2 and water from the egg and the penetration of oxygen into the egg (Eke *et al.*, 2013). In support of this, Samli *et al.* (2005) and Grashorn *et al.* (2016) found a significant increase in air sack size with increased storage time. This parameter was measured visually, which may have influenced the results found in the present study. The difficulty in observing differences between the air sac sizes may also be due to the air sac already enlarging significantly by the time the eggs were one month old.

Lastly, some significant difference was observed between treatments after storage for egg height and shell thickness. Treatment N+2 had a significantly longer or higher egg height in month three than in month one, while treatments N1 and N2 had significantly thicker egg shells in month three. These results were also unconventional due to these parameters being as a result of the physiology of the hen itself when the egg is laid and not as a result of change during storage (Dudusola, 2009; Çağlayan *et al.*, 2009). Some possible explanations for the differences observed may be due to stress experienced during the two periods of egg collection or physiological changes in individual hens. It is therefore possible that hens from the N+2 treatment laid longer eggs from day 16 to 30 of egg collection and the N1 and N2 treatments had thicker shells on day 16 to 30 of egg collection due to the birds taking longer to adjust to the feed. Testing these products over a longer period of time and allowing the birds more time to adapt may improve the results.

4.5 Conclusion

In this chapter, the effect of Nutrifen® and Nutrifen Plus® on egg quality and the storage potential of eggs was evaluated. Generally, both Nutrifen® and Nutrifen Plus® were ineffective in improving egg quality. Of all the parameters measured, Nutrifen® and Nutrifen Plus® seem to have an interesting effect on the yolk colour. The yolk colour is a small but important part of the total egg quality, especially for consumers. N2 and N+2 showed an increase in whiteness of the egg yolk while N1 and N+1 seem to be able to maintain the yellow colour of the egg yolk better during storage. It therefore seems that in this study, the higher inclusion level yielded an increasing negative effect on egg yolk colour. After a storage period of three months, the yolk colour of all treatments deteriorated (became lighter).

On the other hand, interesting results were found for the Nutrifen Plus® diets as they expressed less egg weight loss and a lower egg yolk weight increase compared to that of the Nutrifen® diets and the control. Generally, egg quality for all treatments deteriorated in terms of egg yolk colour, albumen height, and yolk height and albumen weight.

In addition, it is also important to note that although Nutrifen® and Nutrifen Plus® had no effect on the other egg quality parameters when first analysed, the inclusion of these products in the diet did not express any negative effect on egg quality.

The results found need to be supported with similar results; therefore, further investigation is necessary. Investigation should be done to determine if egg yolk colour is decreased with increasing levels of Nutrifen® and if the ingredients in Nutrifen Plus® have the potential to suppress this effect at higher inclusion levels. Future investigation should also consider evaluating the effect of Nutrifen® and Nutrifen Plus® under different environmental conditions and physiological stages of the hen's production. The effect of Nutrifen® and Nutrifen Plus® may be improved by increasing the inclusion levels, selecting a different time for the first analysis, selecting hens of a different age (i.e., older hens may respond better) and adding a stressor (e.g., heat stress) to test the response of stressed hens. The improved colour L value of N2 at month one may be beneficial to consumers and might increase profitability in the short term.

In conclusion, it is evident that Nutrifen® and Nutrifen Plus® had limited impact on the egg quality of layer hens and the storage effects on eggs. In fact, inclusion of these products only stimulates certain parameters. From the outcomes of this research, not enough evidence was generated to verify which product, Nutrifen® or Nutrifen Plus®, is better at improving egg quality and what the ideal concentrations of these products in the diet should be.

4.6 References

Abdouli, H., Haj-Ayed, S., Belhouane, S. & Hcini-Emna, E., 2014. Effect of feeding hens with fenugreek seeds on laying performance, egg quality characteristics, serum and egg yolk cholesterol. J. New. Sci. 1-11.

Abdouli, H., Omri, B. & Tayachi, L., 2014. Effect of whole fenugreek seed before and after its maceration in water on hens' laying performance and egg cholesterol profile. J. New. Sci. 8(4), 28-34.

Adamic, J., Dolezal, M., Mikova, K. & Davidek, J., 2002. Changes in egg volatiles during storage. Czech J. Food. Sci. 20(2), 79-82.

Ahmad, A., Alghamdi, S.S., Mahmood, K. & Afzal, M., 2016. Fenugreek a multipurpose crop: Potentialities and improvements. Saudi J. Biol. Sci. 23, 300-310.

Ahmadi, F. & Rahimi, F., 2011. Factors affecting quality and quantity of egg production in laying hens : A review. World Appl. Sci. J. 12(3), 372-384.

Ahn, D., Kim, S. & Shu, H., 1997. Effect of egg size and strain and age of hens on the solids content of chicken eggs. Poult. Sci. 76(6), 914-919.

Akter, Y., Kasim, A., Omar, H. & Sazili, A.Q., 2014. Effect of storage time and temperature on the quality characteristics of chicken eggs. J. Food. Agric. Environ. 12(3-4), 87-92.

Akyurek, H. & Okur, A.A., 2009. Effect of storage time, temperature and hen age on egg quality in free-range layer hens. Journal of Animal and Veterinary Advances. 8(10), 1953-1958.

Alobaidy, R.N., 2012. Effect of fenugreek seeds and olive leaves ration supplementation on productive and physiological performance of laying breeder hens. Msc Thesis, College of Agriculture and Forestry, University of Mosul.

Alloui, N., Ben-Aksa, S., Alloui, M. & Ibrir, F., 2012. Utilization of fenugreek (*Trigonella Foenum-Graecum*). J. World. Poult. Res. 2(2), 25-27.

Awadein, N.B., Eid, Y.Z. & Abdel-Ghany, F.A., 2010. Effect of dietary supplementation with phytoestrogens sources before sexual maturity on productive performance of mandarah hens. Egypt. Poult. Sci. 30(3), 829-846.

Beardsworth, P. & Hernandez, J., 2004. Yolk colour - An important egg quality attribute. Int. Poult. Prod. 12(5), 17-18.

Blank, I., Lin, J., Devaud, S., Fumeaux, R. & Fay, L.B., 1997. The principal flavor components of fenugreek (*Trigonella foenum-graecum L.*). ACS Symposium Series. 660(3), pp. 12-28.

Çağlayan, T., Alaşahan, S., Kırıkçı, K. & Günlü, A., 2009. Effect of different egg storage periods on some egg quality characteristics and hatchability of partridges (*Alectoris graeca*). Poult. Sci. (November), 1330-1333.

Dudusola, I.O., 2009. Effects of storage methods and length of storage on some quality parameters of japanese quail eggs. Tropicultura. 27(1), 45-48.

Eisen, E.J., Bohren, B.B. & McKean, H.E., 1962. The haugh unit as a measure of egg albumen quality. Poult. Sci. 41(5), 1461-1468.

Eke, M.O., Olaitan, N.I. & Ochefu, J.H., 2013. Effect of storage conditions on the quality attributes of shell (table) eggs. Niger. Food. J. 31(2), 18-24.

El-Kaiaty, A.M., Soliman, A.Z.M. & Hassan, M.S.H., 2002. Combine effect of garlic, fenugreek and black seed on some productive and physiological response of laying hens. Egypt. Poultr. Sci. 22, 147-174.

Fromm, D. & Matrone, G., 1962. A rapid method for evaluating the strength of the vitelline membrane of the hen's egg yolk. Poult. Sci. 41(5), 1516-1521.

Grashorn, M.A. & Simonovic, S., 2009. Wholesome drinking water to prevent watery eggs. World Poult.. 25(6), 18-19.

Grashorn, M., Juergens, A. & Bessei, W., 2016. Effects of storage conditions on egg quality. Univ. Hohenheim, Ger. 50(1), 22-27.

Jin, Y.H., Lee, K.T., Lee, W.I. & Han, Y.K., 2011. Effects of storage temperature and time on the quality of eggs from laying hens at peak production. Asian-Australasian J. Anim. Sci. 24(2), 279-284.

Johnson, A.S. & Merritt, E.S., 1955. Heritability of albumen height and specific gravity of eggs from

White Leghorns and Barred Rocks and the correlations of these traits with egg production. Poult. Sci. 34(3), 578-587.

Jones, D.R. & Musgrove, M.T., 2005. Effects of extended storage on egg quality factors. Poult. Sci. 84(2001), 1774-1777.

Jones, D.R., Tharrington, J.B., Curtis, P.A., Anderson, K.E., Keener, K.M. & Jones, F.T., 2002. Effects of cryogenic cooling of shell eggs on egg quality. Poult. Sci. 81(5), 727-733.

Kirunda, D.F.K. & McKee, S.R., 2000. Relating quality characteristics of aged eggs and fresh eggs to vitelline membrane strength as determined by a texture analyzer. Poult. Sci. 79(8), 1189-1193.

Marusich, W.L. & Bauernfeind, J.C., 1970. Oxycarotenoids in poultry pigmentation: 1. Yolk studies. Poult. Sci. 49(6), 1555-1566.

Menezes, P.C., Lima, E.R., Medeiros, J.P., Oliveira, W.N.K. & Evêncio-Neto, J., 2012. Egg quality of laying hens in different conditions of storage, ages and housing densities. Rev. Bras. Zootec. 41(9), 2064-2069.

Moustafa, E.-K., 2006. Effect of using commercial and natural growth promoters on the performance of commercial laying hens. Egypt. Poultr. Sci. 26, 941-965.

Oleforuh-Okoleh, V. & Eze, J., 2016. Effect of storage period and method on internal egg quality traits of the Nigerian native chicken. Livest. Res. Rural Dev. 28(6), 1-7.

Omri, B., Chalghoumi, R. & Abdouli, H., 2017. Study of the effects of dietary supplementation of linseeds, fenugreek seeds and tomato-pepper mix on laying hen's performances, egg yolk lipids and antioxidants profiles and lipid oxidation status. J. Anim. Sci. Livest. Prod. 1(2:008), 1-9.

Panaite, T.D., Varzaru, I., Criste, R.D., Cornescu, G.M., Ropota, M. & Predescu, C., 2015. Effect of the dietary fenugreek (*Trigonella foenum- graecum*), used as antioxidant in layer diets, on the nutritional quality of the eggs. Arch. Zootech. 18(2), 45-52.

Petrolli, T.G., Albino, L.F.T., Rostagno, H.S., Gomes, P.C., Tavernari, F.D.C. & Balbino, E.M., 2012. Herbal extracts in diets for broilers. R. Bras. Zootec. 41, 1683-1690.

Samli, H.E., Agma, A. & Senkoylu, N., 2005. Effects of storage time and temperature on egg quality in old laying hens. Poult. Sci. 14(1), 548-553.

Silversides, F.G. & Scott, T.A., 2001. Effect of storage and layer age on quality of eggs from two lines of hens. Poult. Sci. 80(8), 1240-1245.

Song, K., Choi, S. & Oh, H., 2000. A comparison of egg quality of pheasant, chukar, quail and guinea fowls. Asian-Aust. J. Anim. Sci. 12(7), 986-990.

Tůmová, E. and Gous, R.M., 2012. Interaction of hen production type, age, and temperature on laying pattern and egg quality. Poultry Science. 91(5),1269-1275.

Walsh, T., Rizk, R. & Brake, J., 1995. Effects of temperature and carbon dioxide on albumen characteristics, weight loss, and early embryonic mortality of long stored hatching eggs. Poult. Sci. 74, 1403-1410.

Yilmaz, A.A. & Bozkurt, Z., 2009. Effects of hen age, storage period and stretch film packaging on internal and external quality traits of table eggs. Sci. Pap. Anim. Sci. Biotechnol. 42(2), 462-469.

Chapter 5: Conclusion

5.1 General conclusion

The animal feed industry is increasingly being placed under pressure to produce food in a more effective and sustainable manner to feed the world's growing population. To address this need, the industry's goal is to move away from the use of synthetic products to improve animal production, thus shifting towards the use of natural resources. Plant extracts, such as the product investigated in this study, need to be evaluated to determine their effects as natural feed additives as they have the potential to improve the production performance of animals, with limited or no negative side effects.

According to the results from this study, Nutrifen® and Nutrifen Plus® seem to have no effect on the overall egg quality. It is however evident from the results that Nutrifen® and Nutrifen Plus® have the potential to affect egg yolk colour. The products also seem to be ineffective in preserving egg quality during storage. Overall, the products exerted no negative effects on egg production for the parameters measured, as most of these parameters were unaffected by treatment rather than negatively affected. Further investigation into the effects of Nutrifen® and Nutrifen Plus® is however necessary.

5.2 Suggestions for future studies

For future studies, it is advised that the interaction between fenugreek and Methylsulfonylmethane (MSM) is tested to better understand the difference in Nutrifen® and Nutrifen Plus®. Furthermore, the inclusion percentage of Nutrifen® and Nutrifen Plus® should be considered as several experiments have shown improved results with inclusion percentages between 1-5% (as opposed to the 0.1% and 0.2% used in this study). There is also the possibility that birds may respond better during stressful periods. Therefore, testing the product on older hens or when hens are in a stressful stage of production (i.e., before post-peak production) may yield better results. Heat stress is another form of stress that can be further explored. In terms of shelf life, it would be interesting to test the effect of storage at different temperatures on the shelf life of the eggs. It may also be worthwhile to test eggs at different storage times. In addition, using an earlier first egg analysis time (i.e., analysed on day of lay) may yield different results regarding the shell life of eggs.