THE EFFECT OF USING COMMERCIAL PROBIOTIC ON THE QUANTITY AND QUALITY OF NATIVE COCKERELS' SEMEN PRODUCTION

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ABSTRACT

This study was conducted to study the effect of using probiotic on the quantity and quality of the native cockerel semen productions. The duration of this study took about 4 weeks. Fifteen cocks were housed in an individual cage, and were given clean water and feed *ad libitum.* Before the cocks were given probiotic, the sperm were collected every week for two weeks and the quantity and quality of the sperm was observed. The cocks were given 5 ml of Probiotics (4.5 g of probiotic mixed with 250 clean water) every day for 2 weeks. The parameters for quality in this experiment is colour, gross motility, and individual motility (%), while the parameters for quantity were the sperm volume of the sperm collected (ml), concentration of the sperm (x 10^9), and live and dead percentage of the sperm and abnormalities of the sperm The result obtained from the experiment showed that the average sperm collected before and after supplementing the chicken with probiotic showed no significant difference ($p>0.05$). The sperm concentration before supplemented with probiotic and after supplementedwith probiotic showed no significant difference ($p>0.05$). The average colour before and after supplementing with probiotic showed no significant difference ($p>0.05$). Average gross motility showed before supplementing the chicken with probiotic and after supplementation showed no significant difference ($p>0.05$). Average percentage of individual motility showed no significant difference before and after supplementing probiotic to the chicken ($p>0.05$). The average live and dead percentage showed no significant difference before and after supplementing with probiotic ($p>0.05$). The average percentage of abnormalities of chicken before and after supplementing the chicken with probiotic showed highly significant difference ($p<0.01$). However further study need to be conducted in order to know the full potential of probiotic.

KESAN PENGGUNAAN PROBIOTIK KOMERSIAL TERHADAP KUANTITI DAN KUALITI PENGEUARAN AIR MANI AYAM JANTAN

ABSTRAK

Kajian ini dijalankan untuk mengkaji kesan penggunaan probiotik kepada kuantiti dan kualiti pengeluaran sperma Ayam. Tempoh pengajian ini mengambil masa kira-kira 4 minggu. Lima belas ayam ditempatkan dalam sangkar individu, dan telah diterima air bersih dan ad libitum makanan. Sebelum ayam sabung diberi probiotik, sperma telah dikumpulkan setiap minggu selama dua minggu dan kuantiti dan kualiti sperma diperhatikan. Ayam sabung diberi 5 ml Probiotik (4.5 g probiotik bercampur dengan air bersih 250) setiap hari selama 2 parameter weeks.The untuk kualiti dalam eksperimen ini adalah warna, motilitas kasar, dan motilitas individu (%), manakala parameter untuk kuantiti adalah jumlah sperma sperma yang dikumpulkan (ml), kepekatan sperma (x 10^9), dan hidup dan peratusan mati sperma dan keabnormalan sperma Hasilnya diperolehi daripada eksperimen menunjukkan bahawa sperma purata dikumpul sebelum dan selepas sebagai tambahan kepada ayam dengan probiotik tidak menunjukkan perbezaan yang signifikan ($p > 0.05$). Kepekatan sperma sebelum menggunakan probiotik dan selepas probiotik tidak menunjukkan perbezaan yang signifikan ($p > 0.05$). Purata warna sebelum dan selepas mengambil probiotik tidak menunjukkan perbezaan yang signifikan ($p > 0.05$). . Purata motiliti kasar menunjukkan sebelum ayam di beri dengan probiotik dan selepas tidak menunjukkan perbezaan yang signifikan ($p > 0.05$). Peratusan purata pergerakan individu tidak menunjukkan perbezaan yang signifikan sebelum dan selepas penggunaan probiotik untuk ayam ($p > 0.05$). Purata peratusan hidup dan mati tidak menunjukkan perbezaan yang signifikan sebelum dan selepas penggunaan probiotik ($p > 0.05$). Peratusan purata keabnormalan ayam sebelum dan selepas penggunaan ayam dengan probiotik menunjukkan perbezaan yang signifikan ($p < 0.01$). Walau bagaimanapun kajian lanjut perlu dilakukan untuk mengetahui potensi penuh probiotik.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATION

µl	Microliter
cfu	Colony- Forming Units
EM	Effective Microorganism
FSA	Faculty of Sustainable Agriculture
GH	Growth Hormone
HCL	Hydrochloric Acid
ml	Mililiter
SD	Standard Deviation
UMS	Universiti Malaysia Sabah

CHAPTER 1

INTRODUCTION

1.1 Introduction

Livestock is one of the major factors of growth in the agricultural sectors. The most rapid growing sectors is the poultry industries sectors. Due to an increase in the demand of poultry by the consumers, the poultry industry have been dynamic and ever expanding sectors in the world. This is due to the demand for a higher protein source, safer consumption is keep on increasing.

The contribution of the poultry sector to national economy is about 7 billion ringgit/year. The share of poultry to livestock value-addition is 55.4 %, while beef 5.8% and pork 6.7%. To ensure that Malaysia are able to self-sustain and became self-sufficient in term of the production of high protein meat, the production of poultry should be increased. Establishment of "Halal Food Hub" to capture the halal meat market in the Middle-East or collaborating with the large exporters of other ASEAN countries since the acceptance of poultry meat to people of all ages, races and religion can also contributes to the national economy and also helps to elevate Malaysian poultry industry.

During intensive growth, the poultry industry have always been confronted with challenges from various diseases. Among this condition, the major economic losses are due to the infectious diseases by infectious pathogen, viruses or bacteria (Lutful, 2009). Hence, there is a high cost on preventive measure. Due to this, there is an increase in the usage of antibiotic in poultry industry.

The residue present from the antibiotic, in human can cause resistance of human flora and pathogenic microbes to those groups of antibiotic (Van den Bogaard and Stobberingh, 2000). Edens (2003) mentioned that with the increase concerns in the resistance of antibiotic, there is increasing interest in finding alternatives to antibiotic in poultry productions. An alternatives approach to sub- therapeutic antibiotics in livestock is the use of probiotic microorganism.

A research done by Tollba *et al.* (2007) shows there is a significant difference in the volume of semen and sperm cell concentration as the level of effective microorganism (EM) is increased in cocks diets. Tollba (2007) also found that the abnormal and dead sperms were significantly decrease in cocks fed with EM and zinc bacitracin compared with those which is not fed with EM and zinc bacitracin. This improvement in semen quality may be due to the improvement in the utilization of protein and minerals absorption.

1.2 Justification

Many developed countries have been using probiotics as an alternatives to antibiotics. The most common microorganism used in probiotic is *Lactobacillus sp.* This microorganism is best known for its therapeutic properties especially in promoting a good intestinal microflora, be it for humans or animals. Based on the positive effects in the previous study on the beneficial effects of probiotics on the fertility and semen quality on chicks and rats, it is proposed to study the effects of probiotic on the quality and quantity of sperm productions in the local or native chicken.

1.3 Objectives

The objectives of this research is:

I. To investigate the effect of commercial probiotic on the quality and quantity of semen productions in the native cockerel.

1.4 Hypothesis

H_o = There is no significant difference in the quality and quantity of semen productions the local or native cockerel when supplemented with probiotic.

H_a = There is a significant difference in the quality and quantity of semen productions in the local or native cockerel when supplemented with probiotic.

CHAPTER 2

LITERATURE REVIEW

2.1 Local Breed

It is widely understood that all populations of domesticated chicken, *Gallus gallus* in the world are from a single ancestor, the Red Jungle Fowl. It has been claimed that other wild species of Gallus might have contributed to all domesticated chicken (Crawford, 1984).The Ayam Kampong, often scuff the soil to search for seed, insects. Owing to these free range and scavenging habits, traditional village poultry are in permanent contact with soil and insects (Pandey *et al.*, 1992). In general the Ayam Kampong is a dual purpose chicken, have a small frame and different plumage colours. Although there existed other varieties of kampung chickens such as the nakedneck, the frizzle, the silkie and the barredfeather varieties among others, their occurrences among the varied group under study were limited to just a few birds (E. Azhan, 1994). The reproduction of Red Jungle Fowl, is a complex mechanism with multiple physiological and environmental factors interacting and contributing to successful copulation and fertilization (Malik *et al.*, 2013).

The taxonomic hierarchy of *Gallus gallus Domesticus:*

Kingdom	: Animalia
Class	: Aves
Order	: Galliformes
Family	: Phasianidae
Genus	: *Gallus*
Species	: *Gallus gallus*
Sub species	: *Gallus gallus domesticus*

Kampung chickens are generally small and are poor performers in terms of growth and egg production (Engku Azahan *et al.,* (1980). Body weights of between 1.1 and 1.5 kg over 4 months have been regularly quoted and they produce about 100 eggs/year. Their poor egg producing capacity is mainly due to the prevalent characteristic of broodiness among the females (Aini, 1990). Over 24 laying weeks, egg production and egg weight of the kampung chickens were inferior to the respective values usually reported for commercial layers (Engku Azahan *et al.,* (1980). Engku Azahan (1983), mentioned that the attribution of the low egg production capacity of the kampung chickens partly to the occurrence of broodiness which might have been responsible for 15% reduction in egg production.

2.2 Basic Anatomy and Physiology of Cockerel's Reproductive Tract.

Studies on the development of the genital system of the domestic fowl and variation in their gonadal size from hatching to sexual maturity have been of great interest to those in the poultry industry and other investigators (Parker and Mc Spadden, 1943). Testes are the major copulatory organs in cockerels. The testes produces sperms, and male sex hormones, testosterone. The male chicken has two testes, located along the chicken's back, near the top of the kidneys. Both of the testes are functional when they reached puberty. The sperms than travelled through vas deferens an then to cloaca. The vas deferens are also main area to store the sperm. By applying pressure in the area, the sperm can be ejaculated. Semen is then released upon sexual stimulation from the vas deferens (Perry, 1981). In the avian reproductive tract, the sperms remain viable at body temperature (41°C). An accurate method to determine the quantity of the sperm produced by a chicken is by measuring the circumference of the testes. The larger the circumference of the testes, the larger the sperm quantity (Senger, 2003)

2.3 Feeding Management

Before farming became more specialized, farmers kept their chicken to scavenge for their food on their own. After the twentieth century, nutritionally complete poultry feed was developed. Modern poultry feed consist of all the important requirement to sustain the growth and production of the chicks. This results in an extensive of feed formulation. The use of supplementation in feeding is common in poultry industries as it is the most rapid growing sectors in agriculture. The supplementation includes hormones, anti-stress, antibiotic and probiotic.

2.4 Supplement used in poultry industry

2.4.1 Hormones

Involvement of pituitary Growth Hormone (GH) is, however strongly suggested by the correlated elevation in blood plasma GH concentration in pullets at the onset of lay (Williams *et al.*, 1986) and around the time of oviposition and ovulation in hens (Harvey *et al.*, 1979). The role for pituitary GH in the function of the ovary is also supported by an increase in the number of small ovarian follicles after GH administration to laying hens (Williams *et al.*, 1986). Similarly, injections of recombinant chicken GH (cGH) to chickens prior to the onset of egg laying resulted in increased ovarian weight about one week before sexual maturity (Hrabia *et al.*, 2011).

2.4.2 Antibiotics

Antibiotic is used to control diseases, prevention and as a therapy for modern livestock animals. It is generally agreed that usage of antimicrobial agents is the most important factor in the selection of resistance in bacteria, and that, in general, a close association exists between the rate of resistance development and the quantities of antimicrobial agents used (McGowanJr, 1983). The residue presence from the antibiotic, in human can cause resistance of human flora and pathogenic microbes to those groups of antibiotic (Van den Bogaard and Stobberingh, 2000). Eden (2003) mentioned that with the increase concerns in the resistance of antibiotic, there is increasing interest in finding alternatives to antibiotic in poultry productions.

2.4.3 Probiotics

Many definition of probiotic have been introduced, starting from Fuller (1989) who defined probiotics as a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance. According to currently adopted definition by Food and Agriculture Organization and World Health Organization (2001), probiotics are, live microorganisms which when administered in adequate amounts confer a health benefits on the host. Consequently, many researchers have partially replaced antibiotics with probiotics as a therapeutic and growth promoting agents. It was reported that probiotics have a good impacts on the poultry performance (Mountzouris *et al.*, 2007; Koenen *et al.*, 2004). Improve microbial balance synthesis of Vitamins (Fuller, 1989), Improve feed consumption (Nahashon *et al.*, 1994). Most of the previous research is on probiotic utilization in poultry focused on multispecies of probiotics.

The mode of action of probiotics in poultry includes maintaining normal intestinal microflora by competitive exclusion and antagonism, altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production, improving feed intake and digestion and neutralizing enterotoxins and stimulating the immune system (Král *et al.*, 2012). Proposed mechanisms of pathogen inhibition by the probiotic microorganisms include competition for nutrients, production of antimicrobial conditions and compounds (volatile fatty acids, low pH and bacteriocins), competition for binding sites on the intestinal epithelium and stimulation of the immune system (Rolfe, 2000).

An excellent probiotic must fulfil some selection criteria such as membership among the normal intestinal microbiota, acid and bile tolerance, gut colonization, production of antimicrobial substances or bacteriocin. Then, it must easily to survive growth on a large scale, retain its viability under storage and field conditions, and be cost effective to use for farm animals (Lan *et al.*, 2003). Probiotic species belonging to Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida and Saccharomyces have a beneficial effect on broiler performance (Kalavathy *et al.*, 2003; Kabir *et al.*, 2004; Santos, 2003).

2.4 Effects of supplementation of Probiotic

2.4.1 Sperms Quality and Quantity

High quality semen should contain a high percentage of vigorous and active sperms which enable them to ascend the female reproductive tract to the site of fertilization which is also necessary to achieve fertilization (Yousef *et al.*, 2003). Probiotics microbes and their supplementation in the poultry diet is suggested to improve the productive performance (Ayed and Ghaoui, 2011 and Dibaji *et al.*, 2012). The enhanced performance of the birds is associated with beneficial effects of the probiotics balancing microflora (Panda *et al.*, 2003, and Dibaji *et al.*, 2012).The results regarding the semen quality in response to probiotics shows a significant increments compared to control. The semen volume and sperm cell concentration were significantly increased as the level of effective microorganism (EM) increase. Abnormal and dead sperms were significantly decreased in cocks fed with EM compared to those who received controlled diets (El-Deep, 2011). On another research done by, Ewuola (2013), on rabbits, his result showed that the right, left and paired testes weights of the rabbits were not significantly different among the dietary treatments, however the epididymal sperm reserves were significantly higher in rabbits fed prebiotic and symbiotic diets than those in the control diet. Probiotic also have been widely used in marine. A research done by Abasali *et al.*, (2011), he found that by using probiotic it can improve the reproductive performance of female platy-fish brood stocks during reproductive stages. A research done by Poutahidis et al., (2014), he observed that, male mice routinely consuming purified lactic acid bacteria originally isolated from human milk had larger testicles and increased serum testosterone levels compared to their age-matched controls. The study also found that it had significantly increased seminiferous tubule cross-sectional profiles and increased spermatogenesis and Leydig cell numbers per testis when compared with matched diet counterparts. Dietary *Lactobacillus reuteri* or other probiotic supplementation may provide an alternative natural approach to prevention of male hypogonadism, absent the controversy and side-effect risks of testosterone replacement therapy (Stanworth and Jones, 2008).

2.4.2 Carcass and Internal Organs

The effects of probiotics on carcass and some internal organs were measured and results shows that feeding broilers with probiotics have significant effects on full carcass weight, empty carcass weight, head weight, neck weight, brain weight and also ileum weight (Roozbeh *et al.*, 2012).The effect of inclusion levels of a 5-bacterial species probiotic in broiler nutrition and results revealed that probiotic inclusion level had a significant effect on broiler growth responses, apparent digestibility coefficients, and cecal microflora composition (Mountzouris *et al.*, 2010).

2.5 Semen collection Technique

Semen is collected by massaging through abdominal and back for a minute. Cocks needs to be trained prior collecting the sperm, this is because they tend to urinate and defecate when stimulated for the first time, until they are adapted to the ritual. The goal of semen collector is to obtain the maximum volume of clean, high quality semen with the minimal amount of handling Bakst and Dymond, 2013). In chickens and turkeys, the abdominal massage techniques (Burrows *et al.*, 1935) involves massaging the cloacal region to achieve phallic tumescence. This is followed by a 'cloacal stroke', a squeezing of the region surrounding the sides of the cloaca to express the semen. Little additional semen can be expressed after two cloacal strokes; additional cloacal strokes may cause damage to the phallic and cloacal regions and contribute to semen contamination (Bakst *et al.*, 2013). The extent of erection is dependent and varies with each individual cockerel (Donoghue and Wishart, 2000).

2.6 Semen Evaluation

Many parameter can be used to evaluate the general quality of cockerel semen and estimate the extent to which semen can be extended such as ejaculated volume, semen concentration, and total number of sperm, sperm motility and morphology (Mosenene, 2009). Semen evaluation are reference to 'viable' sperm simply implies that such sperm possess an intact plasmalemma and are assumed to be functional (Bakst and Dymond, 2013). On a commercial breeder farm, the nigrosin/eosin (N/E) technique is most likely the procedure to be used to determine sperm viability (Bakst *et al.*, 2010).

2.6.1 Ejaculate Volume and Concentration

The cockerel produces between 0.1 ml to 1.5 ml semen per ejaculation. The average ejaculated volume is 0.6 ml. The average volume ejaculated using the abdominal massage technique is approximately 0.25 ml and contains on average 5000x 10^6 sperm/ml (Gordon and Talansky, 2005). Different cockerels of the same species often produces different semen volume at different times (Anderson, 1994). Semen concentration is routinely measure by light scattering in a photometer, colorimeter or spectrophotometer with the optical density correlated with the sperm concentration of samples estimated using haemocytometer. Haemocytometer which was used for counting blood cells was used. It consists of specially designed slides that contain two counting chambers and two dilution pipettes. The counting chambers are 0.1mm and have a ruled area on the bottom of the chambers that is 1.0 mm^2, the square is sub divided into 25 smaller squares (Ameen, 2014)

The concentration of sperm/semen was found using the formula:

$$\text{Concentration} = \frac{Cells\ Counted\ x\ Dilution\ Rate\ x\ Depth\ of\ Hemocytometer}{Number\ of\ Squares\ Counted}$$

2.6.2 Sperm Motility

Sperm motility can be progressive (forward direction) or non-progressive (random movement or oscillations) movement. Sperm motility can be evaluated based on mass motility (MMOT) which is the subjective evaluation of the speed of the movement of group of sperm in 20 µl of semen, ranging from 0 to 9 (Blesbois *et al.*, 2008). Sperm motility assessment is an indicator of the viable of sperm and the quality of the semen sample, generally analysed under the light microscope (x10 magnification) (Mosenene, 2009). Motility evaluated by microscopy has been shown to have little correlation with fertility and simply reveals that the sperm are motile (Baskt and Dymond, 2013)

2.6.3 Live and Dead sperm

The method for assessing sperm morphology against a background stain in dried semen smears is usually used in conjunction with a dye such as eosin, which will only penetrate and stain sperm organelles that have damaged membrane, to enable concurrent assessment of sperm morphology and membrane integrity (Lake and Stewart, 1978). The nigrosin-stain produces a dark background on which the sperm stand out as lightly coloured objects. Normal live sperm exclude the eosin stain and appear white in colour, whereas dead sperm which are those with loss of membrane integrity will take up eosin and appear pinkish in colour.

2.6.3 Sperm Morphology

A normal sperm cell consist of a head, mid-piece and tail portion. Up to 10% of all spermatozoa have observable defects and as such are disadvantaged in terms of fertilising an oocyte (Sadler, 2010). An eosin-nigrosin stain technique to assess the morphology of cockerel semen. This is made by mixing 20 µl of semen with 20 µl of the stain and air dried before being observed under a light microscope (x1000 magnification) (Blesbois *et al.*, 2008). Abnormal shaped semen includes defects on head (bulb, small, enlarged, looped), neck (broken at different angle in relation to head), mid-piece and tail (swelling, looping, partial or totally lacking) (Bloom, 1981).

CHAPTER 3

METHODOLOGY

3.1 Study sites

The study was conducted at the chicken house and the Animal Physiology and Anatomy laboratory of the Faculty of Sustainable Agriculture, University Malaysia Sabah (UMS). The duration of study is about three month, starting from September 2015 and finished on November 2015.

3.2 Animal and Management

Fifteen village cockerels purchased from various places in Sandakan was used as the sperm donor in this study. The cockerel was intensively housed, managed and maintained in an individual cages for the entire period of study. They are allowed to adapt to the environment for one month adaptation prior to onset of semen collection. Throughout the experiments, all the cockerel are fed *ad libitum* with commercial feed formulated by Cargill (United States of America, (USA)).

Figure 3.1 The native village cockerels were managed and maintained in an individual cages

REFERENCES

Abbas Ali,I., Hosseini, H.,Nourani, M.,Khani, S and Alavian, S.2013. Probiotic as a Novel Treatment Strategy against Liver Disease. *Hepat Mon*, **13 (2)**

Aini, I. 1990. Indigenous chicken production in South-east Asia. *World Poultry Science.* **46(01):** 51-58.

Ameen S.A., Opayemi O.S., Ajayi J.A. and Adediwura M.A 2014. Evaluation of Semen Quality of Five different Cockerel Breeds used in poultry industry in Nigeria. *Journal of Environmental Issues Agriculture.* **6(1):**30-36

Anderson, J. 1944. *The semen of animals and its use for artificial insemination.* Edinburgh: Imperial Bureau of Animal Breeding and Genetics.

Ayed, M., and Ghaoui, F. 2011. Efficiency of supplementing *saccharomyces cerevisiae var. ellipsoideus* for improved growth performance and carcass yield in broilers. *Research Opinions In Animal and Veterinary Sciences*, **6(1):** 371-374.

Azahan, E. A. 1994. The red and black-red native chickens of Malaysia. Ayam kampung Malaysia jenis merah dan hitam-merah. *Malaysian Agricultural Research and Development Institute (MARDI) Research journal* **22 :** 73-78.

Azahan, E. and Seet, C.P. 1980. Observations on the performance of some exotic broiler strains in relation to the indigenous 'kampung' chicken. Proc. 5th MSAP Malaysia: 136-144.

Bakst, M. and Dymond, J.S. 2013 Artificial Insemination in poultry. INTECH Open Access publisher.

Bakst, M., Kramer, M., and Long, J. 2010. Effect of Fenbendazole on Turkey Semen Quality. *The Journal Of Applied Poultry Research*, **15(2):** 307-311.

Bakst, M.R. and J.S. Dymond,. 2013. *Artificial Insemination in Poultry.* INTECH Open Access Publisher.

Bassert, J., Thomas, J., and McCurnin, D. 2014. *McCurnin's clinical textbook for veterinary technicians.* St. Louis, Mo.: Saunders/Elsevier.

Bearden, H. and Faquay, J. 2004. Applied animal reproduction. Reston, Virginia. Reston Publication.

Blesbois, E., Grasseau, I., Seigneurin, F., Grasseau-Mignon, S., Saint Jalme, M., and Mialon-Richard, M. 2008. Predictors of semen ability to freezing in chickens. *Theriogenology*, **69:** 252-261.

Bloom, A. 1981. Guidelines for Reproductive studies in Exposed Human Populations. *March Of Dimes Birth Defects Foundations*, **2:** 30-110.

Burrows, W., and Quinn, J. 1937. A Method of Obtaining Spermatozoa from the Domestic Fowl. *Poultry Science*, **14(4):** 251-254.

Cole, H., and Cupps, P. 1969. *Reproduction in domestic animals.* New York: Academic Press.

Dibaji, S., Seidavi, A., and Asadpour, L. 2012. Effect of dietary inclusion of the synbiotic Biomin IMBO on broilers' some blood metabolites. *Research Opinions In Animal and Veterinary Sciences*, **1(2):** 10-13.

Donoghue, A., and Wishart, G. 2000. Storage of poultry semen. *Animal Reproduction Science*, **62(1-3):** 213-232.

Edens, F. 2003. An alternative for antibiotic se in poultry: probiotics. *Rev. Bras. Cienc. Avic.*, **5(2)**

El-Deep, M., Amber, K., and Sayed, M. 2003. Investigating the Effect of probiotics on Chicks Fertility and Semen Quality. *Journal Of Poultry Science*, **90(3):** 195-203.

Etches, R. (1998). A holistic view of poultry science from a reductionist perspective. *British Poultry Science*, *39*(1), 5-10.

Ewuola, E. 2013. Daily sperm production, gonadal and extra gonadal sperm reserves for rabbits fed probiotic and prebiotic supplemented diets. *International Journal of Applied Agriculture.* **9 (1):** 48-53

Fuller, R. 1989. Probiotics in man and animals. *Journal Of Applied Bacteriology*, **66(5):** 365-378.

Gordon, J., and Talansky, B. 2005. Assisted fertilization by zona drilling: A mouse model for correction of oligospermia. *J. Exp. Zool.*, **239(3):** 347-354.

Harvey, S., Scanes, C., Chadwick, A., and Bolton, N. 1979. Growth hormone and prolactin secretion in growing domestic fowl: Influence of sex and breed. *British Poultry Science*, **20(1):** 9-17.

Hazim, J., & Daraji, A. (2012). Effects of Addition of a Probiotic to Roosters Diet on Semen Quality Parameters. *J Probiotic And Prebiotic Res*, **1(1):** 1-4.

Higgins, S., Erf, G., Higgins, J., Henderson, S., Wolfenden, A., Gaona-Ramirez, G., & Hargis, B. (2007). Effect of Probiotic Treatment in Broiler Chicks on Intestinal Macrophage Numbers and Phagocytosis of Salmonella Enteritidis by Abdominal Exudate Cells. *Poultry Science*, **86(11):** 2315-2321.

Hrabia, A., Sechman, A., Gertler, A., and Rzasa, J. 2011. Effect of growth hormone on steroid content, proliferation and apoptosis in the chicken ovary during sexual maturation. *Cell Tissue Res*, **345(1):** 191-202.

Kabir, S., Rahman, M., Rahman, M., Rahman, M., and Ahmed, S. 2004. The Dynamics of Probiotics on Growth Performance and Immune Response in Broilers. *International Journal Of Poultry Science*, **3(5):** 361-364.

Kalavathy, R., Abdullah, N., Jalaludin, S., and Ho, Y. 2003. Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *British Poultry Science*, **44(1):** 139-144.

Khan, R., Rahman, Z., Javed, I., and Muhammad, F. 2013. Effect of vitamins, probiotics and protein on semen traits in post-molt male broiler breeders. *Animal Reproduction Science*, **135(1-4):** 85-90.

King, L., Kirby, J., Froman, D., Sonstegard, T., Harry, D., & Darden, J. et al. 2000. Efficacy of sperm mobility assessment in commercial flocks and the relationships of sperm mobility and insemination dose with fertility in turkeys. *Poultry Science*, **79(12):** 1797-1802.

Koenen, M., Kramer, J., van der Hulst, R., Heres, L., Jeurissen, S., and Boersma, W. 2004. Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. *British Poultry Science*, **45(3):** 355-366.

Kral, M. Angeloviaubici, M., and Mraizovai, L. 2015. Applications of probiotics in Poultry. *Animal Science and Biotechnology*, **1(45):** 235-356.

Kunavongkrit, A., Kesdangsokonwut, S., and Tummaruk, P. 2004. Relationships among specific reasons for culling, reproductive data, and gross morphology of the genital tracts in culled due to reproductive failure in Thailand. *Theriogenology* **71(2):**369-375

Kuster, C., Singer, R and Althouse, G. 2004.Determining sample size for the morphological assesmentof sperm. *Theriogenology* **61 (4):** 691-703

Lake, P.E and Stewart J.M 1978. Artificial Insemination in Poultry. Ministry of Agriculture , Fisheries and Food.

Lan, P., Binh, L., and Benno, Y. 2003. Impact of two probiotic Lactobacillus strains feeding on fecal lactobacilli and weight gains in chicken. *The Journal Of General And Applied Microbiology*, **49(1):** 29-36.

Malik, M., Ansari, M., Hussain, I., Rakha, B., Akhter, S., and Blesbois, E. 2013. Semen characteristics of the Indian Red Jungle Fowl (Gallus gallus murghi). *Eur J Wildl Res*, **61(3):** 379-386.

McGowan, J. 1983. Antimicrobial Resistance in Hospital Organisms and Its Relation to Antibiotic Use. *Clinical Infectious Diseases*, **5(6):** 1033-1048.

Mosenene, T.M.B. 2009. Characterization and cryopreservation of semen of four South African chicken breeds. Msc, Faculty of Natural and Agricultural Science, Department of Animal, Wildlife and Grassland Science, University of Free State Bloemfontein.

Mountzouris, K., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G., and Fegeros, K. 2007. Evaluation of the Efficacy of a Probiotic Containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus Strains in Promoting Broiler Performance and Modulating Cecal Microflora Composition and Metabolic Activities. *Poultry Science*, **86(2):** 309-317.

Mountzouris, P., and James, L. 2010. The Effect of Probiotics on Carcass and Internal Organs of Broilers. *Poultry Science*, **(89):** 58-67.

Nahashon, S., Nakaue, H., And Mirosh, L. 1994. Production Variables and Nutrient Retention in Single Comb White Leghorn Laying Pullets Fed Diets Supplemented with Direct-Fed Microbials. *Poultry Science*, **73(11):** 1699-1711.

Omeje, S and Marire, B. 1990. Evaluation of the semen characteristics of adult cocks of different genetic backgrounds. *Therionology*, **34(6):**1111-1118.

Panda, A., Rama Rao, S., Raju, M., and Sharma, S. 2007. Effect of probiotic (Lactobacillus sporogenes) feeding on egg production and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders. *Journal Of The Science Of Food And Agriculture*, **88(1):** 43-47.

Parker, J., and McSpadden, B. 1943. Seasonal Variation in Semen Production in Domestic Fowls. *Poultry Science*, **22(2):** 142-147.

Poutahidis,T.,Springer, A.and Levkovich,T. 2014. Probiotic microbes sustain youthful serum testosterone levels and testicular size in aging mice. *Plos one*,**9(1):**e84887

Rolfe, D. 2000. The role of probiotic cultures in the control of gastrointestinal health. *The Journal of Nutrition* **(130):**396-402.

Roozbeh, S., Mehran, N., Faramin, J., and Hamed, K. 2012. The effect of probiotics on carcass and internal organs of broilers. *Annals Of Biological Research*, *3*(12), 5475-5477.

Sadler, K., and Yuca, O. 2001. Postmeiotic Unfertilized Starfish Eggs Die by Apoptosis. *Developmental Biology*, **237(1):** 29-44.

Santos, J., Storch, O., and Gil-turnes, C. 2003. *Bacillus cereus var. toyoii* and *Saccharomyces boulardii* increased feed efficiency in broilers infected with *Salmonella enteritidis*. *British Poultry Science*, **46(4):** 494-497.

Senger, R. 2003. Sperm competition selects beyond testes size in birds. *Animal Reproduction Science*, **80(12):** 7505.

Stanworth,F. and Jones, M. 2008. Feeding and stress interact through the serotonin 2C receptor in developing mice. *Physiology and Behaviour*, **94(4):** 569-579

Tollba, A., Adam, M., and Rahman, S. 2007. Improvement of Fayoumi Laying Hens performance Under Hot Climate Condition. *Egypt Poultry Science*, **1(27):** 1-20.

Van den Bogaard, A. 2000. Epidemiology of resistance to antibiotics Links between animals and humans. *International Journal Of Antimicrobial Agents*, **14(4):** 327-335.

Williams, J., Harvey, S., and Leclercq, B. 1986. Plasma Levels of Luteinizing Hormone, Growth Hormone, and Estradiol from Six Weeks of Age to Sexual Maturity in Two Lines of Chickens Selected for Low or High Abdominal Fat Content. *Poultry Science*, **65(9)**

World Health Organization.2002. Guidelines for the evaluation of probiotics in food. London Ontario, Canada.

Yousef, M., Abdallah, G., and Kamel, K. 2003. Effect of ascorbic acid and Vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Animal Reproduction Science*, **76(1-2):** 99-111.