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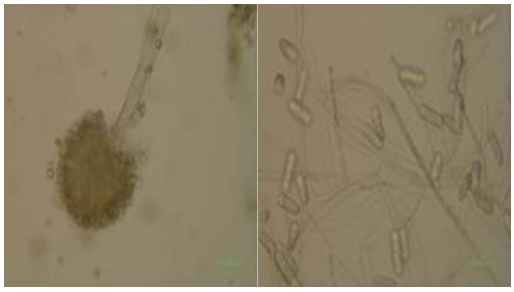

Biological Control of Mycotoxins: An Update.

Fapohunda SO, Esan AO and Anjorin TS

World Vet. J. 7(4): 117-127, 2017;
pii:S232245681700015-7

ABSTRACT
Biological control, or rather the deployment of living organisms in an effort to arrest the growth and development of another organism, is a hot topic in mycotoxin studies. Confirmed environmental inclemency and increasing cases of resistance, brought about by the use of chemical applications have invited the development of natural and better alternatives. Many candidates from bacteria through yeasts to fungi have been exploited to control mycotoxin-producing fungi with appreciable success. This review takes a critical look at the development and harvest the reaction of crop and livestock farmers and other stakeholders and, concludes that the bio- control of mycotoxins is a field with a promising future, in spite of a few research gaps that have to be filled.
Key words: Biological control, Crop, Food safety, Mycotoxin

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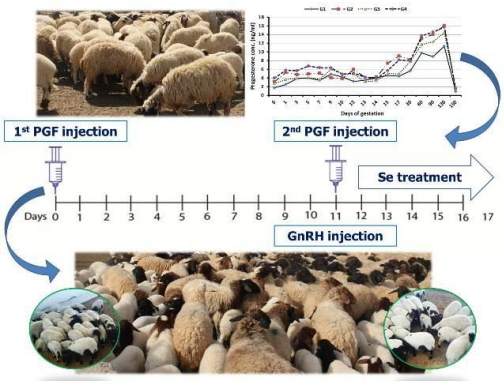

Improving Reproductive and Productive Efficiency of Barki Sheep by using GnRH and Selenium.

Farrag B, El-Hawy AS, El-Bassiony MF.

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ABSTRACT
The present study was conducted to investigate the effects of hormonal treatment (GnRH) and/or selenium (Se) supplementation on improving the reproductive and the productive efficiency of Barki ewes. Sixty-two Barki ewes were assigned into four groups. G1 group (15 ewes) severed as a control group only fed CFM, without Se supplementation or GnRH injection, G2 (16 ewes) was estrus-synchronized with double injections of PGF2α 11 days apart and intramuscularly injected with 2 ml of GnRH at day 11 and were fed CFM without Se, G3 (15 ewes) received double injections of PGF2α 11 days apart and were supplemented with Se, while G4 was (16 ewes) estrus-synchronized with double injections of PGF2α 11 days apart and intramuscularly injected with 2 ml of GnRH at day 11 and supplemented with Se. Reproductive parameters, milk yield and composition as well as animal weights were recorded. Progesterone hormone concentration was also measured. The result indicated that conception and lambing rates were higher ($P < 0.05$) in G4 (93.75%) as compared to G1 (80 %) while G2 and G3 recorded 87.5 and 86.66%, respectively. The numbers of lambs born alive and weaned were higher in all treated groups than the control group. Mortality rate from birth to weaning had increased in the control group than treated ones. Milk yield, milk fat and protein had increased insignificantly in Se groups (G3 and G4). The data of lambs birth weight and average daily gain showed significant increases in G3 and G4, while the weaning weight had not been affected with values being similar. GnRH administration increased plasma progesterone concentration compared with the controls. In conclusion, GnRH administration and Se supplementation improved reproductive parameters and milk yield and composition as well as their lambs' weights, probably through its beneficial effect on embryo survival by enhancing luteal function.
Keywords: Reproductive efficiency, Barki sheep, Selenium, GnRH.

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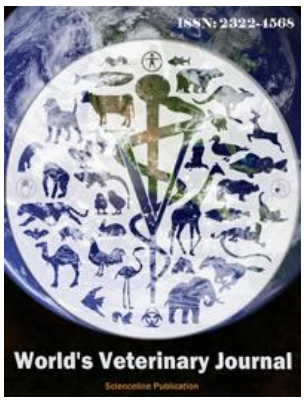

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Biological Control of Mycotoxins: An Update

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ABSTRACT

Biological control, or rather the deployment of living organisms in an effort to arrest the growth and development of another organism, is a hot topic in mycotoxin studies. Confirmed environmental inclemency and increasing cases of resistance, brought about by the use of chemical applications have invited the development of natural and better alternatives. Many candidates from bacteria through yeasts to fungi have been exploited to control mycotoxin-producing fungi with appreciable success. This review takes a critical look at the development and harvest the reaction of crop and livestock farmers and other stakeholders and, concludes that the bio- control of mycotoxins is a field with a promising future, in spite of a few research gaps that have to be filled.

Key words: Biological control, Crop, Food safety, Mycotoxin

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INTRODUCTION

Mycotoxins are toxic secondary metabolites that are produced by some pathogenic fungi on crops, whether raw or processed, in farm, transit or store, they are capable of inciting morbidities in man and animals under appropriate conditions. Targets of this contamination include cassava, maize, millet, groundnut, rice, sorghum, melon, wheat, soybean, beans, milk and a variety of spices and vended foods that are intended for human consumption (Kayode et al., 2013, Rubert et al., 2013, Anjorin et al., 2016, Abdi-Mohammed et al., 2016).

Mycotoxins can be classified according totheir structure and effect. On the basis of their chemical structure they can be grouped as Aflatoxins (*Aspergillus flavus*), Ochratoxin-OTA (*Aspergillus westerdijkiae),* Patulin (*Aspergillus* and *Penicillium*) and DON, ZEA, Fumonisin . However, by taking their health impact into consideration, it should be noted that they can be carcinogenic (Afl, OTA, Fumonisin,) neurotoxic (Fumonisin) nephrotoxic (OTA), dermatotoxic (Trichothecenes DON), immunosuppressive (AF, OTA, DON) and embryotoxic, teratogenic (AF, ZEA). The health impacts have been well documented among livestock (Table 1). Feedmillers and other animal care enthusiasts sometimes introduce binders and other interventions to reduce the adverse effect of mycotoxins on their stock. On the bases of surveillance, health impact as well as the need for interventions they are broadly categorized as **'**regulated**'** and **'**non-regulated'.

High level of broken grains and nuts, length of time stored, damage by insects and mites, degree of invasion before purchase and inadequate harvesting, drying and storage practices are some of the predisposing factors that make the grain susceptible to mycotoxigenic fungi. Contamination can occur at any given stage in the overall food and feed value chain – pre-harvest, at harvest, and in the storage. However, delivery of the fungus fighters can only be achieved in pre harvest and store. Dietary mycotoxin was found to incite oxidative stress in mice (Hou et al., 2013) and rat (Vasatkova et

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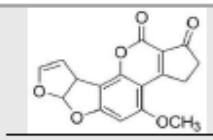
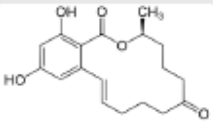
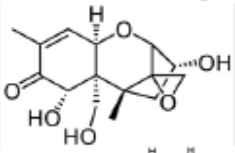
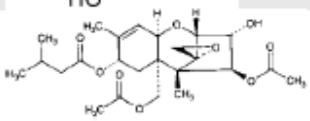
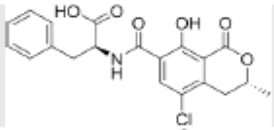
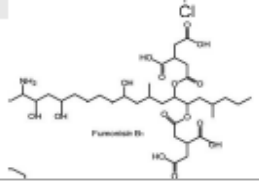
al., 2009). In humans there is a suggested link between aflatoxin B_1 and viral load in HIV positive cases (Jolly et al., 2013).

Table 1. Mycotoxins and their effects on livestock

Mycotoxins	Chemical Structure	Productivity Loss	Immuno Toxicity	Frequently Related Clinical Signs	Main Affected Organ System	Animal Affected
Aflatoxins		+++++	+++++	Hepatitis, poor response to vaccination, unspecific infections, increased susceptibility to diseases	Liver, Kidney, immune system	Dairy cattle, poults, pigs
Zearalenone		+++++	++	Hyperestrogenism, reproductive disorders	Reproductive tract -mainly female	Pigs, poults
Deoxynivalenol		+++++	+++++	Feed refusal, vomiting	Central nervous system, GUT epithelium, liver, immune system	Pigs, dairy, cattle
T-2 toxin		+++++	+++++	Oral and epithelial lesion, loss of appetite	GUT epithelium, liver, immune system	Horses, pigs, poults
Ochratoxin A		+++++	+++++	Nefritis (kidney damage- enlarged kidney) nepatitis	Kidney, liver, immune system	Poults, cattle, rats
fumonisins		+++++	+++	Porcine Pulmonary, Edema (PPE) Equine Leukoencephalomalacia (ELEM)	Lungs and heart (pig), central nervous system (horse), liver, immune system	Beef, cattles, pigs, horses

Source-https://www.impextraco.com/products/animal-protection/power-protexion-power-mycotoxins-eliminator , as modified by authors.(accessed 19 August 2017).

Although some half-hearted success has been recorded in chemical control (Belli et al., 2007; Valero et al., 2007). The regularly observed and high number of fungal strains resistant to chemical agents and the noxious impact of chemicals in the control of mycotoxins make them increasingly unattractive. Recently, there has been a global call for the wholesale embrace of organic and green agriculture, a major component of which is non-chemical applications for soil improvement as well as pest and herb control. Although phytochemicals could hold a promise in fungal control in Africa (Anjorin et al., 2014) this product of bio -prospecting can at present can only attract a measured trust. This article hereby has attempted to review of the most regulated mycotoxins biocontrol agents and the possible challenges that are inherent in adopting these strategies.

Mycotoxins control agents

Mycotoxin biocontrol agent, was first employed in the US, with success on a million - acre of susceptible crops are treated annually (Bandyopadhyay et al., 2016) The major success of patented ones is the intervention done by Cotty (1994) on cotton seeds, Dorner et al. (1999) on maize and rice and Dorner et al. (2003) on peanuts. For example, the production of norsorolinic acid, a biosynthetic intermediate involved in an early step of the aflatoxin biosynthetic pathway, can be inhibited thereby reducing the mRNA level of *afIR*, a gene encoding a key regulatory protein necessary for the expression of aflaxoxin-biosynthetic enzymes (Jermnak et al., 2013).

Biological control strategies have been employed with some success in using fungi, bacteria and yeasts (Tsitsigiannis et al., 2012). Some of them are being marketed under different trade names (Table 2). Qualities expected in a biological control agent include: genetic stability, efficacy at low concentrations and against a wide range of pathogens, simple nutritional requirements, survival in adverse environmental conditions, growth on cheap substrates in fermenters, lack of pathogenicity for the host plant , no production of metabolites potentially toxic to humans, resistance to the most frequently used pesticides and compatibility with other chemical and physical treatments (Spadaro and Gullino, 2004). A criticism of fungus-based bio-pesticide is that they act slowly and, therefore, give limited protection to crops. Clearly, more aggressive strains of fungal BCAs can be sought i.e. those which work more quickly and require a lower inoculum. Factors that determine pathogen virulence (virulence determinants) should be identified and used in strain selection and quality control. Fortunately, some progress has been made in this area regarding enzymes and metabolites having been identified as important virulence or antagonistic determinants.

Applications of fungal bio-control agents are usually timed to coincide with frequent rainfall and high soil moisture. When drought conditions prevail after application, the active ingredient, the fungi, remain alive on the carrier grains and

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are expected to sporulate when the conditions are conducive. Sporulation has also been observed under drought conditions with low soil moisture on carrier grains that are lodged under the plant canopy with the canopy both protecting the carrier and providing humidity for night sporulation. Screening and natural selection are essential preliminary ingredients in biocontrol. This ensures that the fighter 'organism' has the requisite traits to gain the most effective result out of the screened lot.

Aflatoxins

Aflatoxins are a notorious group on crops like grains, pulses and many others. They also attack animal products. The problem of aflatoxin lies particularly in the sub Saharan Africa is that is caused by a lack of awareness and economic challenges. These two factors combine effectively and result in many cases of unrecorded morbidities as well as rejections of export crops by the European Union. Morbidity can come as liver cancer and immuno-suppression. Hell and Mutegi (2011) reported that the leading self-sustaining, commercially effective, environment-friendly technology to reduce aflatoxin accumulation of crops is by using atoxigenic isolates of *A. flavus* as biocontrol agents for the displacement of aflatoxigenic fungi. This innovative technology is already widely used in the US, and field testing of the product in Nigeria has produced extremely positive results as aflatoxin contamination of maize and groundnut is reported to have been consistently reduced by 80-90% (Bandyopadhyay and Cotty, 2013). The success of bio-control products as bio-pesticides in the US has encouraged researchers of the International Institute of Tropical Agriculture (IITA) and the USDA-ARS to develop, adapt, and improve the bio-control approach for African agro-ecosystems. Collaboration among IITA, USDA-ARS, and several partners has resulted in both successful adaptation of the bio-control technology for use on maize and groundnuts in various African nations and the development of several bio-control products under the trade name 'Aflasafe' (Atehnkeng et al., 2014).

Afla-Guard based on the nontoxigenic strain *A. flavus* NRRL21882 for aflatoxin control in the USA, on corn (field, sweet, and popcorn) and peanuts (Isakeit, 2012) has been very effective. In Nigeria, Aflasafe TM with an indigenous non-toxigenic strain *A flavus* to act as the bio-pesticide formalize is the attractive equivalent. Successful attempts at reducing aflatoxin through non toxigenic strains were also recently carried out (Alaniz Zanon et al., 2016; Mallikarjunaiah et al., 2016).

Bacillus, Pseudomonas and *Bulkholderia* strains completely inhibit *A. flavus* growth (Palumbo et al., 2006). Full-faeces-sourced *Stenotrophomonas maltophilia*, and a few other microbes were also able to confer remediation strategies. Specifically, Shifa et al. (2016) had also succeeded in reducing the aflatoxin load when there was challenge with *Bacillus subtilis* in a groundnut matrix The multiple genetic mechanisms which incite aflatoxin production (Kelly et al., 2014) are often the target of attack.

Crop specificity is a significant factorin determining a candidate biopesticide. For example, there are no known cotton varieties that demonstrate enhanced resistance to *A. flavus* infection and aflatoxin contamination. Therefore, transgenic approaches are being undertaken in cotton fields that utilize genes encoding antifungal/anti-aflatoxin factors from maize and other sources to counter fungal infection and toxin production (Cary et al., 2011).

The timing and environment of release are a few factors that need to be observed before determining the efficacy of a bio-control candidate. Garber and Cotty (2006) had reported that spore production of AF36 had reduced significantly after AF36 product was exposed to six herbicides, Buctril, Bueno, Caparol, Gramoxone, Prowl and Roundup, at the recommended use rates, which indicated that non-toxigenic strains should be applied, post herbicide administration. The same year, Pitt and Hocking (2006) recommended that the application of non-toxigenic strains to soil should be delayed until soil temperature reaches at least 20^{o}C. All these factors are aimed at ensuring that the non-toxigenic strains reach high population levels when the threat of crop infection is at its greatest (Yin et al., 2008).

It has been reported that aflasafe products provide excellent protection from aflatoxin accumulation both before and after harvest and throughout the value chain (Bandyopadhyay et al., 2016). To date, fungal bio-control products have been registered for use in Nigeria as aflasafe in 2014, in Kenya as aflasafe KE01 in 2015, and Senegal/The Gambia as aflasafe SN01 in 2016. Research is currently underway to secure registration of tailor-made aflasafe products in Burkina Faso, Burundi, Ghana, Malawi, Mozambique, Rwanda, Tanzania, Uganda, and Zambia (Grace et al., 2015). Most of the popular biological control products on mycotoxins are pre-harvest interventions. In Nigeria and sub-Saharan Africa, aflatoxin is still considered and reported as a postharvest challenge.

Fumonisin

This *Fusarium* mycotoxin is dangerous as it incites hepatotoxicity and nephrotoxicity in most animals it's in contact with. It is associated with leucoencephalomalacy in horses, pulmonary edema in pigs and can be related to oesophageal cancer in humans. The biological control of fumonisin has involved an extensive use of bacteria and fungi.

For example, maize seed treatment with *Bacillus amyloliquefaciens* and *Enterobacter hormaechei* may improve the quality of maize grains obtained at harvest by reducing their toxin content (Perreira et al., 2010). Rhizobacteria, particularly those belonging to the *Pseudomonas* and *Bacillus* species were reported to produce significant reduction in *Fusarium verticilloides* and fumonisin B production (Cavaglleri et al., 2004). The use of *Bacillus amyloliquefaciens* and *Microbacterium oleavarans* to reduce fumonisin in maize was reported (Sartori et al., 2013) just as *Trichoderma harzianum* T16 and T23 on *F. moniliforme* and Fumonism B1 and B2 (Altinook, 2009). Also, the inhibitory effect of *Trichoderma* species on fumonisin–producing *Fusarium* has been traced to a combination of antibiosis through production of volatile compounds, extracellular enzymes and antibiotics (Alberts et al., 2016). Many *Trichoderma* species are also known to produce Microbe-Associated Molecular Patterns (MAMPs) which respond by reflex to the presence of foreign body by expressing anti-microbials. Through these mechanisms, many Generally Recognized as

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Safe, GRAS, species like *T. viride* effectively reduce *F. verticillioides* growth and fumonisin production both *in vitro* and *in planta* (Yates et al., 1999).

Microbial transformation of fumonisin to less toxic derivatives have been carried out by using organisms like *Exophiala spinifera* ATCC 74269, *Rhinocladiella atrovirens* ATCC 74270, bacterium ATCC 55552, and *Sphingopyxis macrogoltabida* MTA144 (Heinl et al.,2010). The bacterium, Serenade, (*B. subtilis*), has been successfully used to reduce fumonisin and aflatoxin (Formenti et al., 2012).

In the rural subsistence farming clusters, simple, practical, and culturally acceptable methods like hand-sorting, washing, and de-hulling are very effective in fumonisin reduction (Alberts et al., 2016). It is on record that while mechanical de-hulling is effective, mechanical shelling is not; in fact it leads to more *Fusarium* invasion and enhance fumonisin contamination (Fandohan et al., 2006). A consortium of mycoparasitic bacteria and some fungi was used to challenge *Fusarium verticillioides* and fumonisin production and was found to have been successful in toxin reduction (Samsudin and Magan, 2015).

Ochratoxin

Ochratoxin A is another important mycotoxin present in coffee, spices, and cocoa. *Aspergillus westerdjikiae* and *A. ochraceus* are usual sources. OTA affects rat mid brain (Wilk-Zasadna and Minta, 2009) and reproductive disorders due to mycotoxin effects are often reported in farm animal species. Health impacts on humans and livestock include nephrotoxicity and general reduction in productivity. The total removal of OTA from foods and feeds is not attainable till date. Kapetanakou et al. (2012) reported the importance of yeasts and bacteria in OTA degradation. Experimental *Beauveria bassiana* ITEM-1559 was reported to be a valid bio-insecticide against the moth *L. botrana* and that grape moth bio-control is a strategy to reduce OTA contamination (Cozzi et al., 2013).

Yeast isolates, *Issatchenkia arientalis, Metschnikowia pulcherrima, Issatchenkiaterricola* and *Candida incommninis*have been observed to reduce the *A. carbonarium* and *A. niger* colonization on grape berry (Bleve et al., 2006). This is due principally to the biology and no -toxin properties of yeasts (Ponsone et al., 2012). These two fungi were earlier successfully bio-controlled by the use of yeast strains (Ponsone et al., 2011). Strains of *Aureobasidium pullulans* could drastically reduce the OTA production in wine grapes (de Felice et al., 2008). Some mycotoxins have a combination effect which needs to be studied in order to serve as guide in their control. For example, there is the possible synergistic effect between Ochratoxin A (OTA) and other mycotoxins, such as Penicillic Acid (PeA) and Fumonisin B1 (FB_1), contributing to this nephropathy (Stoev, 2008). Shi et al. (2014) had reported that *B. subtilis* CW 14. had inhibited the growth of the OTA-producing species *Aspergillus achraceus* 3.4412 and *Aspergillus carbonarius*. The bacterium according to the researchers was able to both prevent OTA contamination and degrade OTA in crops. Out of many wild yeasts tested for OTA reduction, *Pichia anomala* CCMA0148 and *Saccharomyces cerevisiae* CCMA0159 provided the greatest inhibitory influence on toxin producing strains (de Souza et al., 2017).

The suppressing effect of *Streptomyces aureofaciens* on OTA producing *A. niger* in grapeis on record (Haggag and Abdal, 2012). Also, Ponsone et al. (2012) investigated two epiphytics strains of yeast *Lanchancea thermotolerans* that were able to control the growth and OTA accumulation of ochratoxigenic fungi both "*in vitro*" and "*in situ*". Yeasts are noted fungicides. This toxin is also known to be controlled by the yeast *Trichosporon mycatoxinivorans*, a species that can also detoxify the zearalenone (Vekiru et al., 2010). Environmental factors like temperature and humidity can affect the efficacy of bio-control agents on *Aspergillus carbonarius* and *A. niger* (Leong et al., 2006) and particularly when yeast *Metschnikowia pulcherrima* LS16 and two strains of the yeast-like fungus *Aureobasidium pullulans* LS30 and AU34-2-were investigated by De-Curtis et al. (2012) against infection by *A. carbonarius* and OTA accumulation in wine grape berries. *Saccharomyces cerevisiae* was also successful on *A. ochraceus* and OTA in coffee (Velmourougane et al., 2011). The yeast bullet impact was also tested on *Penicillium nordicum* and OTA production when a consortium of yeasts belonging to *D.hansenii, D. maramus, C. famata, C. zeylanoides* and *H. burtonii* species, was individually screened for antagonistic activity against a toxigenic strain of *P.nordicum* and inhibition of OTA biosynthesis (Virgili et al., 2012) *C. zeylanoides* and *H. burtonii* were the most effective and had their activity was enhanced by the presence of sodium chloride.

A trip into the mechanism of action of OTA degradation by biological means was taken by Shi et al. (2014). Using the 16SrRNA gene sequence it was revealed that *B. subtilis* CW 14 could inhibit the growth of the OTA-producing species *Aspergillus ochraceus* 3.4412 and *Aspergillus carbonarius*, with inhibition rates of 33.0 and 33.3% respectively. An interesting dimension was introduced when it was observed that, using high-performance liquid chromatography, the cell-free supernatant degraded 97.6% of OTA after 24h or incubation at 30 ^{o}C and no degradation products were produced. It could only suggest that OTA was an ingredient of survival and growth for this bacterium.

Yeasts are considered one of the most potent biocontrol agents due to their biology and non-toxic properties. Epiphytic yeasts are the major component of the microbial community on the surface of grape berries and they are evolutionarily adapted to this ecological niche. Nowadays, several yeast species included in different genera are considered as potential bio-control agents to control both growth of ochratoxigenic *Aspergillus* species and OTA accumulation (Ponsone et al., 2012).

Deoxynivalenol (DON)

DON, sometimes thought to be a temperate contaminant has its presence also in African countries. Since this notorious field mycotoxin, is distributed throughout the kernels, with higher content in the outer skin, milling can also be effective in reducing the DON levels of wheat-based foods, if bran and shorts are removed before thermal cooking. Field conditions that guarantee the moisture level of 22 and 25 percent such as delayed harvesting will lead to *Fusarium* growth and toxin contamination. DON is water-soluble and cooking with larger amounts of water lowers DON content

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in products such as spaghetti and noodles. During baking or heating, DON is partially degraded to DON-related chemicals, (Kushiro, 2008). There has been a confirmation of antibody mediated reduction in *Fusarium* mycotoxins (Hu et al., 2008).

Patulin (PAT)

The mycotoxin, patulin (4-hydroxy-4H-furo (3,2c]pyran-2[6H]-one) was first isolated from *Aspergillus clavatus*. It is prevalent and well disseminated in apple juice and pome fruits generally. The level 50ppb has been set at the USA, the world largest consumer of apple juice its mechanism of action, though not well known it is believed to be linked with yeast growth inhibition (Iwahashi et al., 2006). The combination of *Rhodosporidum kratochvilovae* LS11 (originally named *Rhodotorula glutinis*) and *Cryptococcus laurentii* LS28 and some low dose fungicides reduced the level of patulin in apples (Lima et al., 2011). Biocontrol yeasts *Rhodasphoridium kratochvilovae* strain LS11 (Castoria et al., 2011); *Rhodotorula glutinis* (Wright et al., 2008) are ready candidates of patulin reduction. Ianiri et al. (2013) while working on *Sporobolomces sp.* strain IAM 13481, a basidiomycetes yeast, investigated the genetic approach to patulin reduction and linked this to the mechanism of resistance of the mycotoxin. Manning et al. (2013) believed that clean grounded corn DON fed at high dose of 5ppm (5mg DON/Kg) to catfish had actually conferred protection fromexposure to the pathogenic bacterium *Edwardsiella ictaluri.* It strengthens their immune system and allows alternative use of such contaminated corn instead of discard.

ZEARALENONE (ZEA)

This *Fusarium* toxin was noted to incite a drastic reduction in productivity of livestock has been controlled by the use of *Aspergillus niger* (Sun et al., 2014). According to them, rats administered with contaminated corn steep liquor treated with the strain FS10 culture filtrate had shownto have entailed a significantly less severe liver and kidney damage, and organ index values were comparable to the non-ZEN-exposed control.

Many *Rhizopus* strains, including *R. stolonifer, R. oryzae* and *R. microspores* were found to completely degrade ZEN (Varga et al., 2005). A substantial biotransformation of zearalenone by the two fungal genera *Aspergillus* and *Rhizopus* was reported by Brodehl et al. (2014). Biotransformation normally could result in less toxic derivatives as in identification and characterization of a lactonohydrolase enzyme in fungus *Clonostachys rosea* which converts ZEN to a less estrogenic compound (Takahashi-Ando et al., 2005; Kosawang et al., 2013). The metabolism of zearalenone was also recorded by using *Gliocladium roseum* which gave 80-90% yields in less toxic residue (Saleh and Yusuf, 1988). Bacteria also have a role to play as *Lactobacillus acidophilus* had the ability to protect the liver, kidney, and uterus from the toxicity of zearalenone in albino rats (Ali et al., 2015).

Table 2. Biocontrol products for mycotoxins

Product/Trade name	Microbial agent	Food commodity	Manufacturer/distributor
AF36	*Aspergillus flavus* AF36	Corn and cotton	Arizona Cotton Research and protection Council USA
Afla-guard	*A flavus* Strain NRRL21882	Peanuts and corn	Syngenta Crop Protection, USA
AQ-10 biofungicide	*Ampelomyces quisqualts* Cesah ex Schlechtendahl	Apple, grapes, strawberries, tomatoes and cucurbitus	Ecogen. Inc. USA
Aspire	*Candida olephila* strain 1-182	Apple, pear and citrus	Ecogen. Inc. USA
Biosave 10LP. 110	*Pseudomonas syringae* (stain 10 LP, 110)	Apples, pear and citrus, cherries and potatoes	Eco Science Corporation, USA
Blight Ban A 506	*Pseudomonas fluorescence* A. 506	Apple, pear, strawberries and potatoes	Nu Farm lnc. USA
Contrans WG, Intercept WG	*Coniothytrium minitans* Campbell	Onion	Prohyta Biologischer, Germany
Messenger Rhio-plus	*Erwinia amylovora* (Burnll) Winslow et al *Bacillus subtilis* FZB 24	Vegetables Potatoes and other vegetables	EDEN Bioscience Corporation, USA KFZB Biotechnick, Germany
Serenade	B subtilis	Apple, pear, grapes and vegetables	AGRO Quess lnc. USA
Aflasafe	Mixture of four *Aspergillus flavus* Atoxigenic VCGs La3279, Kal6127, Og0222 and La3304	Maize and groundnut	IITA Business Incubation Platform, Ibadan Nigeria

Source: (Sharma et al., 2009; Bandyophadyay 2015, Personal communication)

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Challenges of commercializing fungal Biocontrol Agents (BCAs)

Successful usage and adoption of these bio- control strategies are often faced with some challenges. There is still restricted adoption of biocontrol agents (BCAs) in crop protection in developing countries. There are no strong incentives to develop these agents and/or discourage chemical pesticides.

The infrastructure which facilitates the transfer of new technologies and research knowledge to the "end user" (i.e. farmer) in most developing countries is either absent or has does not function.

Work on the biological control of citrinin received a boost when Abd Allah et al. (2005) had observed a successful control using *Trichoderma harmatum*. The matrix was rice. A genetic approach was earlier exploited by Ammar et al. (2000), when one special *pet-ts* mutant had been identified that exhibited a high sensitivity against citrinin. The genetic system of yeast allowed the isolation of the respective wild-type gene. Generally, the genetic influence of yeast *Pichia* on mycotoxin producing species of *Penicillium* had reported interactions among mould species under stress conditions. The yeast *Pichia anomala* (J121) is handy here as it inhibited growth of *P. roqueforti* in grain stored in malfunctioning airtight storage systems (Boysen et al., 2000).

Econo-ecological factors and biological control

Farmers in developing countries are hardly rewarded for guaranteeing reduced mycotoxins in their crop produce.. The visible detection of mould is a preliminary but unreliable indicator of mycotoxin contamination. For rural farmers, many incidents of mycotoxins on crops go un-noticed and therefore undetected by some cultural practices. Biological control will have to rest on the confirmation of the occurrence and the access to available intervention strategies. The cost of the available chemical control is unaffordable by these set of crop and livestock producers. In large scale commercial production toxin reduction cost can also build up thus adding to the operational cost of production which will be passed on to the consumers. The organic farming option is also gaining more ground in Africa with many NGOs willing to partner with producers of bio-pesticides for mycotoxin control, the two might be the cheap twin –option that pull wholesome consumption along the food value chain One principle on biological control is the fungus-fight, where a fungus known to be genic is brought to challenge a toxigenic one in a manner that the latter overcrowds the former and hinder its growth and possible mycotoxin production.

Generally, in carrying out the function of 'fungus-fight', bio-pesticides still need to respond to ecological factors A strain of mould with the genetic potential to produce a particular mycotoxin may not do so under all circumstances. There must be enough nutrients to encourage sustained mould growth and the level of mycotoxin production would in part be influenced by the nutrients available to the mould. Substrate effect is critical to toxin production, e.g., there is a high proportion of toxin-producing strains of *A. flavus* isolated from peanuts and cottonseed than from rice or sorghum. It has also been found that strains of ochratoxin and citrinin-producing *P. viridicatum* isolated from meat were more unstable than those isolated from grain and rapidly lost toxin-producing ability. Field fungi like *Fusarium* and *Alternaria* contaminate grains before or during harvest. The storage fungi (e.g. *Penicillium* and *Aspergillus*) are capable of growing at lower water content than the field fungi and they tend to contaminate the grains in silos and other storage places. It is known that aflatoxin - production is favoured by the prolonged end of season drought and associated elevated temperatures (Kabaluk et al., 2010).

The subject of biological control continues to wallow in academic scrutiny, as a few questions keep returning to the surface. The issues are however, to assist in making bio- control as effective and reliable as initially thought out. What is the mode of action of biopesticides with reference to the chemical state of the mycotoxins? This question is apt for all intervention steps. However, does a biological control agent have an advantage whether the mycotoxin is in free or modified state? Modification can come from diverse sources e.g. thermal as in DON (Beyer et al., 2009), hydrolysed fumonisin, UV- induced in OTA and citrinin (Schmidt-Heydt et al., 2012) or treatment with sodium bisulphite (Beyer et al., 2010). As mycotoxicology is grappling with the issue of 'modified' (formerly 'masked' as proposed by Rychlik et al., 2014) mycotoxin, it is expected that bio-control will play a key role in addressing this group. Climate change with the attendant possibilities on mutation is also critical to the success of bio-control (Battilani et al., 2016).

If the overall intention of bio-control is to achieve bioremediation and bio- recovery of the matrix, then the basic assumption will be that the latter is confirmed toxin-laden *ab initio*. But what happens if this was not true after all? For post-harvest—(storage and processing) and feed- bound bio-pesticides, the suspicion of mycotoxin presence can be confirmed

Therefore, pre-application assessment of the target crop and environment may be critical, in determining the efficacy, just as sample preparation and sampling are to laboratory analysis of mycotoxins. Questions like 'was there any incidence of deoxynivalenone, fumonisin, aflatoxin or ochratoxin A in that particular field?'; was the experience of farmers traceable to a mycotoxin?'.

Unlike in the EU, harmonized regulations for the adoption of biological control are not implemented in developing countries. The large number of countries with porous borders and the weak development of quarantine facilities on this continent pose particular challenges for implementing Africa-wide biological control programs. With an increase in the application of BCAs for pest and disease control, many countries are now adopting regulations for the registration and release of agents. A common set of regulations could play a dramatic role in enhancing the use of BCAs and eco-friendly pest and disease control especially in developing countries. Though strict observation of guidelines on Environmental Risk Assessment and national regulations may delay implementation of biological control Guidelines, these are necessary in order to enhance the existing standard and promote quality.

Mycotoxin-producing fungi are ubiquitous in soil and in crop produce throughout developing countries especially in tropical Africa (Fapohunda et al., 2012; Probst et al., 2014). The large majority of farmers, rural and urban dwellers consume the crop produce as foods without the possibility of monitoring for mycotoxin due to the absence of monitoring

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mechanisms (Shephard, 2008) leading to high chances of human exposure. It is recalled that the European Union (EU) generally seems to be having issues with some Nigerian agricultural commodities like melon, beans, peanuts and *ogbono* due to their mycotoxin levels that are above permissible limits. During the 2006 outbreak investigation in Kenya, a portable screening tool was adapted for rapid assessment of aflatoxin contamination in maize in the rural village setting. This tool was used to identify households with contaminated maize, a key step in the maize-replacement effort (Saha, 2009).

The high investment profile in bio-control for relatively poor farmers, possibility of time –related mutation and subsequent pathogenic tendencies, coupled with inconclusive environmental impact report may invite suspicion of its overall safety and economics. The influence of environmental and cultural practices the survival of *Aspergillus* or any mycotoxigenic fungus cannot also be over looked (Jaime and Cotty, 2010) Recently, the post-release environmental fate of atoxigenic *A .flavus* is attracting research attention in the USA, it is believed that the outcome will resolve the fear earlier expressed (Abbas and Weaver, 2011).

Farmers in developing countries are hardly rewarded for guaranteeing reduced mycotoxins in their crop produce. The organic farming option is also gaining ground in Africa with many NGOs willing to partners with producers of biopesticides for mycotoxin control, the two might be the cheap twin –option that pulls wholesome consumption along the food value chain.

CONCLUSION

Biological control agents should be made affordable to farmers and compounded in a manner that makes products easy and safe to handle. The efficacy of microbe-based control agents may be enhanced by selection of more efficient strains, gene manipulations, combination of a number of strains of microorganism and combination of synergistically acting other bio-products. At the global produce market, the demand for safe and productive crop is high.

The combination of Good Agricultural Practices (GAP), physical, chemical and biological control, and strong emphasis on awareness still remains the best panacea. Simple cultural practices like good storage practices, proper ventilation, washing and picking out/separating of contaminated seeds are still the accessible cheap attractions to an average farmer and housewife processors and vendors of agrochemicals who have informal education with zero knowledge of toxigenic mould infestations and mycotoxins. Much of the mycotoxin challenge can be solved through awareness and cultural practices. It is advised that, on a scale of expenditure, these affordable interventions should have about 70% of all funds released by donor or investors. This is the only way a genuine result can be attained at the lower level of production chain, characterized by people who are economically challenged and lack the required knowledge. Biological control is still viewed with caution and circumspection, particularly with the environmental impact assessment over time being regarded as inconclusive. It will be a 'hit-breakthrough' for humanity if any pre-harvest intervention confers protection on postharvest/stored crop irrespective of where or how it is stored or processed.

Certainly, some departments in the biological control project may need further scrutiny. In spite of that, the biological control of mycotoxins is an intervention with a bright future.

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Author`s contribution

Fapohunda initiated the review, and actively participated in the write up. He is the corresponding author. Esan contributed immensely to the writing of the manuscript through fresh suggestions. Anjorin wrote extensively and was involved at every stage of the review. Financing of the publication was by all authors.

Competing interest

The authors have no competing interest in preparing the manuscript

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Improving Reproductive and Productive Efficiency of Barki Sheep by using GnRH and Selenium

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ABSTRACT

The present study was conducted to investigate the effects of hormonal treatment (GnRH) and/or selenium (Se) supplementation on improving the reproductive and the productive efficiency of Barki ewes. Sixty-two Barki ewes were assigned into four groups. G1 group (15 ewes) severed as a control group only fed CFM, without Se supplementation or GnRH injection, G2 (16 ewes) was estrus-synchronized with double injections of $PGF_2\alpha$ 11 days apart and intramuscularly injected with 2 ml of GnRH at day 11 and were fed CFM without Se, G3 (15 ewes) received double injections of $PGF_2\alpha$ 11 days apart and were supplemented with Se, while G4 was (16 ewes) estrus-synchronized with double injections of $PGF_2\alpha$ 11 days apart and intramuscularly injected with 2 ml of GnRH at day 11 and supplemented with Se. Reproductive parameters, milk yield and composition as well as animal weights were recorded. Progesterone hormone concentration was also measured. The result indicated that conception and lambing rates were higher ($P<0.05$) in G4 (93.75%) as compared to G1 (80 %) while G2 and G3 recorded 87.5 and 86.66%, respectively. The numbers of lambs born alive and weaned were higher in all treated groups than the control group. Mortality rate from birth to weaning had increased in the control group than treated ones. Milk yield, milk fat and protein had increased insignificantly in Se groups (G3 and G4). The data of lambs birth weight and average daily gain showed significant increases in G3 and G4, while the weaning weight had not been affected with values being similar. GnRH administration increased plasma progesterone concentration compared with the controls. In conclusion, GnRH administration and Se supplementation improved reproductive parameters and milk yield and composition as well as their lambs' weights, probably through its beneficial effect on embryo survival by enhancing luteal function.

Key words: Reproductive efficiency, Barki sheep, Selenium, GnRH.

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INTRODUCTION

The efficiency of production in sheep depends heavily on the reproductive performance of females. The incidence of twin births in Barki ewes is low and considerable economic advantages would be accrued from the making available of effective methods, concerning the increase of the reproductive rate in this breed.

Selenium deficiency plays a role in numerous economically important livestock diseases, problems that include impaired fertility, abortion, retained placenta and neonatal weakness (McDowell et al., 1996). Administration of Se improves daily weight gain of lambs and reproductive performance in ewes (Gabryszuk and Klewiec, 2002). The organic selenium from selenomethionine (Se-Met) or Se - enriched yeast is an ideal additive because animals absorb and retain it more than inorganic selenium (Ortman and Pehrson, 1997).

Hormonal treatment to control ovulation and reproduction is a requirement for successful breeding and increasing the number of pregnant females as well (Motlomelo et al., 2002; Branimira et al., 2017), resulting in a short breeding period and more uniform newborn crop (Husein and Kridli 2003). Synchronization of estrus is a valuable management tool that has been successfully employed to enhance reproductive performance, particularly in ruminants (Kusina et al., 2000 and Ambrose et al., 2014). Some studies have successfully used Gonadotropin releasing hormone (GnRH) treatment in combination with progestagens, gonadotropins and prostaglandin (Husein and Kridli, 2003; Reyna et al.,

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2007; Beilby et al., 2009; Hashem et al., 2015). GnRH agonist treatment at the day of mating causes a surge of LH to be released, resulting in an improved luteal function and luteinization of developing follicles (Beck et al., 1996), which may stimulate conceptus growth (Khan et al., 2007). GnRH has been used to induce ovulation and to shorten the time to initiate estrus in conjunction with progestagen (Jabbour and Evans, 1991) or $PGF_2\alpha$ (Naqvi and Gulyani, 1998).

Therefore, the objective of the present study was to attempt to improve the reproductive and the productive performance efficiency by injection of GnRH or/and added organic selenium to Barki ewes under the arid conditions of the North Western Coast of Egypt.

MATERIALS AND METHODS

This study was carried out during the period from June 2015 to February 2016 at the Animal Production Unit in the Sustainable Development Center for Matrouh Resources, Matrouh Governorate, belongs to the Desert Research Center in the North Western Coast of Egypt. The purpose of the present study was to evaluate the effectiveness of GnRH and or organic Selenium for improvement reproductive and productive performance of Barki ewes.

Ethical approval

This experiment was performed according to all ethics and animal rights (Desert Research Center). As much as this work had considering all rules and regulations in conformity with the European Union Directive for the protection of experimental animals (2010/63/EU).

Animals and Management

Sixty two adult Barki ewes (3-5 years old with an average body weight of 41.2 ± 2.13 kg) were assigned randomly into four groups (G1 and G3 N=15 ewes, while G2 and G4 had N=16 ewes).Group 1 (G1) served as control and was not treated with hormonal treatment and was fed CFM without Se. Groups 2, 3 and 4 estrus-synchronized with double injections of prostaglandin $F_2\alpha$ (Estrumate, Coopers Animal Health LTD, Berkhamsted-England). Each ml of Estrumate contained 250 mg Cloprostenol Acetate), 11 days apart. On day 11, both G2 and G4 were intramuscularly injected with 2 ml of GnRH (Receptal, Intervet International, B.V. Manufactured in the European Union). Groups G3 and G4 were fed diets supplemented with Se, while G2 was fed a diet without Se.

All groups were fed a concentrate feed mixture (Table 1) and berseem (*Trifolium alexandrnum)* hay to cover their requirements through the different physiological stages according to Kearl (1982). Animals were housed separately in shaded pens and were clinically healthy and free from internal and external parasites. Fresh water was available to all groups daily.

Table 1. Ingredients percentage and proximate chemical analysis of experimental ration (on 100% DM basis)

Ingredients	Diet	Chemical composition (%)	Diet
Yellow corn	50	Dry Matter (DM)	91.1
Wheat bran	20.5	Crude Protein (CP)	14.5
Cotton seed meal	20	Crude Fiber (CF)	7.4
Soya bean	6	Ether extract (EE)	3.1
Limestone	1.5	Nitrogen free extract (NFE)	71.8
Mineral mixture	1	Ash	3.0
Sodium chloride	1	--	--

Se (ALKOSEL) added 100 g/ton for G2 and G3.

ALKOSEL is a natural product made from the wall of yeast *Saccharmyces* and contains 2000-2400 ppm of selenium in the form of selenomethionine (98% organic selenium). 100 g ALKOSEL was mixed with 2 kg feed to obtain a homogenous mixture of ALKOSEL, which was then added to a 1 ton diet and mixed to obtain homogeneity.

Hand mating started after the second injection of $PGF_2\alpha$ within 5 days duration, while animals in control group were left for 34 days during the mating season (equal to 2 estrous cycles). Four fertile rams were allowed to rotate among different ewe groups to avoid a ram/group confounding effect.

Milk samples (50 ml) were taken biweekly from ewes within the respective groups during the 12 weeks lactation period, in plastic bags and kept under -20 ^{0}C for further analysis. Milk yield was determined biweekly from lambing up to the 12 weeks lactation period, through the complete hand milking of the udder after having fasted the lambs for 12 hours, for two consecutive days once at night and the next at morning to cover 24 hours. The chemical composition of milk in terms of fat, protein, lactose, total solids and solids and not fat was determined using milk scan (Bently-Belguim).

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Blood samples (5 ml) from all groups were withdrawn from the jugular vein into EDTA tubes. The samples were collected from each ewe that had mated at that day, days 1, 3, 5, 7, 9, 11, 13, 15, and 17 then monthly until parturition. Blood samples were centrifuged at 3000 rpm for 20 minutes for the separation of serum and were kept at -20 ^{o}C until further analysis. Progesterone hormone was quantified by ELISA method using a BIOS kit provided by Chemux BioScience Corporation, 385 Oyster Point Blvd Suite 5-6, South San Francisco, CA 94080, USA. The standard curve ranged between 0-50 ng/ml. The sensitivity of the curve was 0.2 ng/ml.

Birth and weaning weights at day 90 after parturition and average daily gain were recorded for lambs born to ewes of this study.

Statistical procedure

Data of the reproductive traits were analyzed using "all or non traits" according to Snedecor and Chocran (1980), while milk yield and composition, lambs weight and P_4 concentrations were analyzed using General Linear Model Procedure (SAS, 2004). The design was one way analysis. The model was as follows:

$Y_{ij} = \mu + T_i + e_{ij}$

Where, Y_{ij} = any observation of j^{th} animal within i^{th} treatment;

μ = overall mean;

T_i = effect of i^{th} treatment (i = 1-4);

e_{ij} = experimental error.

Duncan Multiple Range Test (Duncan, 1955) was used to test the level of significance among means ($P<0.05$).

RESULTS AND DISCUSSION

Reproductive parameters

In this study, GnRH and/or Se improved all reproductive traits and had reduced mortality rate compared to none treated animals. Reproductive parameters (rates of conception, lambing, weaning and mortality) have been presented in Table 2. Ewes of the control group (G1) had recorded the lowest ($P<0.05$) conception and lambing rates (80%) while G4 had recorded the highest rate (93.75%). G2 and G3 recorded 87.5 and 86.66%, respectively. These results indicated that hormonal treatment and dietary Se supplementation have a positive effect on conception and lambing rates. The improvement of conception and lambing rates in treated groups might be related to the enhanced follicular development and ovulation (Titi et al., 2008).

Weaning rate showed the same trend of conception and lambing rates. On the other hand, mortality rate from birth to weaning in lambs born to those ewes that had received Se was lower than their counterparts that had no Se supplementation (7.69 and 6.25% in groups 3 and 4 vs. 16.66 and 13.33% in groups 1 and 2).These results are in agreement with those reported by Pilarczyk et al. (2004), who observed that lamb mortality in the experimental group that had received sodium selenite was lower than the control group (9.2% vs.12.1%). Several studies reported better conception rate with synchronization protocols based on GnRH and $PGF_2\alpha$ than those based on $PGF_2\alpha$ (Stevenson and Pully, 2012; Youssefi et al., 2013; Branimira et al., 2015). Moreover, Ahmadi and Mirzaei (2016) found that twining rate of GnRH treated ewes was significantly higher than untreated ewes, while Cinar et al. (2017) found that estrus synchronization protocols including $PGF_2\alpha$ and GnRH hadn't significantly affected conception, kidding and twinning rates in hair goats. Finch and Turner (1996) showed that the diet inclusion of Se in concentrate above of requirement level was associated with improvements in animal performance and immune function.

No still births and abortion cases were found. The number of lambs born alive and weaned was higher in G4 followed by G2 and G3, while the control group had the lowest value (Table 2). This is in agreement with the previous findings of Cam et al. (2002), Khan et al. (2007) and Lashari and Tasawar (2007) who observed a positive effect of GnRH administration on the day of mating on embryo survival in sheep and cows. Also, the results of this study provided evidence that GnRH causes ovulation and the formation of accessory CLs. A higher number of CLs were observed in slaughter ewes (at day 25 of pregnancy) which were given 2 ml of GnRH at the day of mating after 2 doses of PGF2α (Lashari and Tasawar, 2010).

Titi et al. (2010) studied the oestrus synchronization in Awassi ewes and Damascus does using progestagen sponges and eCG (S), GnRH, and PGF2α (GP) and GnRH, progestagen and PGF2α (GSP). They found that the greatest lambing rate ($P<0.05$) in ewes was shown in the GSP group compared with the control (no treated) and S groups, while GP group was intermediate. However, similar kidding rates were observed among treatments in Damascus does. Husein and Kridli (2003) reported that the luteal tissue that forms as a result of the GnRH administration is responsive to $PGF_2\alpha$ and is capable of undergoing luteolysis.

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Table 2. Reproductive performance of the different experimental groups

Items	G1	G2	G3	G4	*P* Value
No. of ewes joined	15	16	15	16	
No. of ewes conceived	12	14	13	15	
Conception rate (%)	80^{b}	87.5^{ab}	86.66^{ab}	93.75^{a}	0.053
No. of ewes aborted	0	0	0	0	
No. of ewes barren	3	2	2	1	
No. of still births	0	0	0	0	
No. of ewes lambed	12	14	13	15	
Lambing rate (%)	80^{b}	87.5^{ab}	86.66^{ab}	93.75^{a}	0.053
No. of lamb born alive	12	15	13	16	
No. of lamb weaned	10	13	12	15	
Weaning rate (%)	83.33^{b}	86.66^{ab}	92.30^{a}	93.75^{a}	0.058
No. of mortal lambs	2	2	1	1	
Lamb mortality (%)	16.66^{a}	13.33^{a}	7.69^{b}	6.25^{b}	0.043
Litter size %	100	107.14	100	106.66	0.143

G1; control group, G2; $PGF_2\alpha$ and GnRH without Se, G3; $PGF_2\alpha$ and Se, G4; $PGF_2\alpha$, GnRH and Se. Conception rate = number of ewes conceived / number of ewes mated x100. Lambing rate = number of ewes lambed / number of ewes mated.Weaning rate = number of lambs weaned / number of lambs born. Litter size = number of lambs born / number of ewe lambed. Mortality (%) = lambs born alive - lambs weaned / lambs born alivex100.

On the other side, Koyuncu and Yerlikaya (2007) reported that Se and Se plus vitamin E had significant positive effects on incidence of oestrus, fertility and prolificacy in ewes, which supported our results. Positive effects of Se on fertility were also observed by Koyuncu et al. (2006). Se supplementation enhances the level of Se and may indirectly improve livestock performance (Sobiech and Kuleta, 2002), possibly by strengthening the immunity of the animals (Milad et al., 2001). Moreover, Se supplementation during early and mid-pregnancy reduced the time taken for their lambs to stand and improves the immune status of the lambs, resulting in lower prenatal mortality and higher growth rates to weaning (Munoz et al., 2009). In addition, considered that increasing twining in ewes in response to Se supplementation could often be attributed to the increased live weight and hence to ovulation rate (Hemingawy, 2003).

Basini and Tamanini (2000) demonstrated that Se may modulate ovarian granulosa cells proliferation and estradiol 17β synthesis in vitro, affecting ovulation and the number of live embryos. The results of reproductive parameters of this study are in agreement with Cook and Green (2007), who reported that the incidence of retained placental, abortion, early embryonic death and ovarian cysts increase in Se deficient in cows and that Se supplementation overcomes some forms of infertility in ewes. Moreover, the inclusion of Se in the maternal diet improves the embryo viability and growth of the progeny (Pappas et al., 2008).

Whereas, Gabryszuk and Kiewiec (2002) found that Se plus vitamin E did not increase the reproductive performance in younger ewes. Moreover, parental Se supplementation of pregnant ewes between 15-35 days after mating results in a reduced embryonic survival rate (Niekerk et al., 1996).

Milk yield and composition

Ewes fed diets supplemented with Se had a milk yield (G3 and G4) higher than the other two groups (G1 and G2) with differences being insignificant (Table 3), which indicated that hormonal treatments did not affect milk yield. These results are in agreement with those reported by Pauselli et, al. (2004) who found that milk yield was higher in animals treated with vitamin E and Se but animals treated with vitamin E only seemed to have no effect on milk production or mammary health which confirm the role of Se and vitamin E in the host's defense against mastitis. On the other hand, ewes fed diets supplemented with Se showed the highest ($P<0.05$) milk fat percentage (4.70 and 4.23%) for G3 and G4, respectively, compared to G2 (3.45%), while the milk fat of the control group was near to those of Se groups (4.17%). Results of milk protein and total solids had the same trend found in milk fat (Table 3). No significant differences were observed in milk lactose and solids not fat.

Othmane et al. (2002) reported an increase of milk yield with higher litter size, while milk contents decreased or were not affected**.** Moreover, litter size had significantly affected milk and protein yield, while no significant effect was found for fat yield and milk contents in East Friesian sheep (Horstick, 2001). Better rumen parameters for organic Se supplemented to diets that may improve productive efficiency in farm animals (Arzola et al., 2008).

Tufarelli and laudadi (2011) concluded that dietary supplementation with Se had led to an increase in milk production as well as milk fat and protein of dairy Jonica goats which confirmed our results. Lacetera et al. (1999)

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studied the effect of injecting 5 ml of selenium on day 30th before lambing (BL) and 2.5 ml on day 30 before lambing and 2.5 ml at lambing (BLL) on immune function and milk production of Sardinian ewes. They found that ewes belonging to BL and BLL groups and the number of their offspring's were significantly higher ($P<0.01$) glutathione peroxidase activity of erythrocytes (GSHpx-E), moreover, milk yield increased ($P<0.05$) in two treated groups as compared to none treated group. The positive correlation between GSH_{PX}-E and milk production was reported for dairy goats (Atroshi et al., 1985) and cows (Lacetera et al., 1996). For dairy cows, Niki et al., (1991) supposed that the well-known protective role of GSHpx on membrane integrity might represent at least one of the mechanisms through which Se can increase milk production. Wang et al. (2009) concluded that Se supplementation of cows ration in the form of Se yeast positively influenced milk production through the positive influence of Se yeast on fermentation in the rumen, which in turn resulted in the enhanced digestibility of nutrients contained in rations.

Table 3. Effect of treatments on milk yield and composition of Barki ewes during experimental period

Item	G1	G2	G3	G4	SEM	*P* Value
Milk yield (ml)	497	469	545	525	91.9	0.798
Milk composition						
Fat (%)	4.17^{ab}	3.45^{b}	4.70^{a}	4.23^{ab}	0.245	0.026
Protein (%)	5.34^{b}	5.47^{b}	5.62^{ab}	5.93^{a}	0.137	0.053
Lactose (%)	5.01	4.90	4.94	5.09	0.112	0.067
Total solids (%)	15.88^{ab}	15.20^{b}	16.48^{a}	15.72^{ab}	0.287	0.054
Solids not fat (%)	11.56	11.74	11.78	11.54	0.206	0.085

G1; control group, G2; $PGF_2\alpha$ and GnRH without Se, G3; $PGF_2\alpha$ and Se, G4; $PGF_2\alpha$, GnRH and Se

Lambs growth

Effect of Se supplementation and/or hormonal treatment on lambs birth weight (BW), weaning weight (WW) and average daily gain (ADG) are shown in Table 4. The main finding was that BW and ADG had increased ($P<0.05$) in animals treated with Se supplementation (G3 and G4), while WW had insignificant increase in Se groups (15.64 and 15.68 Kg for G3 and G4 vs. 14.80 and 14.72 Kg for G1 and G2, respectively). Data obtained in this study are similar to those reported by Gabryszuk and Klewiec (2002) who found that Se given to ewes increased the average daily weight gain of their lambs from birth to 28 days of age. Similar results were obtained by Langlands et al. (1991), who observed higher weight gains in lambs born to ewes that had received Se-enriched feed than in lambs born to untreated ones.

Moreover, Koyuncu and Yerlikaya (2007) found that Se or Se plus vitamin E led to an increase in BW, WW and ADG as compared to control group. These positive responses are variable depending on species, physiological state and chemical form of Se (Rooke et al., 2004). The differences between lamb weights may be related to milk production. The amount of milk per lamb is indispensable in first weeks of life, because it is the main nutrient source for proper growth, development and health (Godfrey et al., 1997).

Table 4. Productive performance of Barki lambs of the different experimental groups

Experimental groups / Performance	G1	G2	G3	G4	SEM	*P* Value
Birth weight, kg	3.28^{ab}	3.05^{b}	3.63^{a}	3.64^{a}	0.089	0.040
Weaning weight, kg	14.80	14.72	15.68	15.64	0.197	0.156
Average daily gain, g	122^{b}	130^{ab}	140^{a}	131^{a}	18	0.051

G1; control group, G2; $PGF_2\alpha$ and GnRH without Se, G3; $PGF_2\alpha$ and Se, G4; $PGF_2\alpha$, GnRH and Se

Progesterone profile

Plasma progesterone concentration of Barki ewes in control and other treated groups are presented in Fig. 1. Progesterone profile during estrus cycle and pregnancy period was found to have followed the normal pattern. Mean plasma progesterone concentration increased significantly with advancing pregnancy in all groups from day 2 after mating due to the presence of active CL. Mean plasma progesterone concentrations of ewes treated with GnRH (G2 and G4) tended to be insignificantly higher as compared to control group. Plasma progesterone levels exhibiting peak at the 120th day of pregnancy and will gradually decline thereafter till parturition. Abd-Elaziz et al. (2004) noted that progesterone level increased during pregnancy, reached its highest level during days between 130-140 and then declined

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during the last 10 days before parturition. A similar result of progesterone profiling has been observed by Lashari and Tasawar (2010).

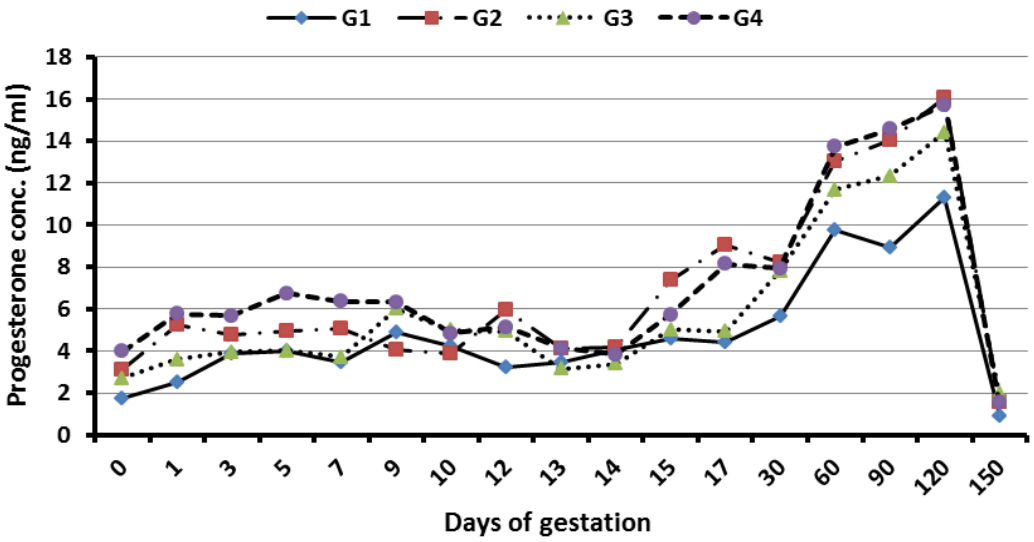

Figure 1. Progesterone profile of the experimental groups during pregnancy period in Barki ewes. (G1: control group; G2: $PGF_2\alpha$ and GnRH without Se; G3: $PGF_2\alpha$ and Se; G4: $PGF_2\alpha$, GnRH and Se).

The increase in progesterone concentrations after GnRH injection suggests that GnRH through LH release might be able to provide luteotrophic stimulation to CL, which could explain the increase in CL weight observed in the ewes given this treatment (Farin et al., 1988). This luteotrophic stimulation might be related to a form of conversion of small luteal cells to large luteal cells, which then secrete higher concentrations of progesterone hormone (Farin et al., 1988).

CONCLUSION

The results of the present study showed that GnRH administration and Se supplementation had improved the reproductive and the productive efficiency of Barki ewes as well as their lambs. We believe that these results are useful and good benefits would be accrued from the availability of such methods to improve the reproductive efficiency of the Barki sheep.

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Competing interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article. The authors declare that they have no competing interests.

Author's contribution

Dr. Bahaa Farrag designed the experiment, collected data; Dr. Ahmed Sobhy El-Hawy helped in statistical analysis, tabulation of experimental data and article writing; while, Dr. Moharram Fouad El-Bassiony helped in laboratory analyses, manuscript writing, commenting and approval. All authors have read and approved the final manuscript.

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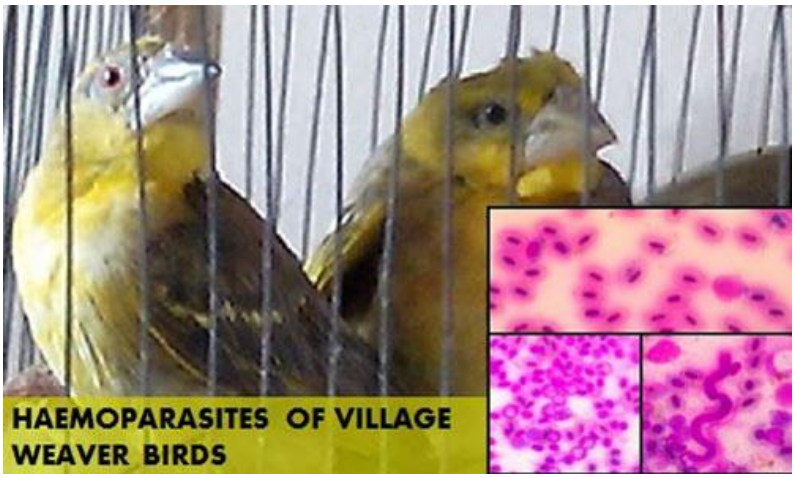

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4. Names of authors and title of journals, published in non-latin alphabets should be transliterated in English.

5. A sample of standard reference is " 1th Author surname A, 2th Author surname B , 3th Author surname C. 2013. Article title should be regular and 7 pt . *World Vet. J.,* Add No. of Volume (Add No. of Issue): 00-00."
6. Just full title of a journal is acceptable in references.

-Examples (at the text)*:*

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001).

--Examples (at References section)*:*

a) For journal:

Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. Journal of Dairy Science, 83: 1635-1647.

Kareem SK (2001). Response of albino rats to dietary level of mango cake. J. Agric. Res. Dev. pp 31-38. Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. African Journal of Biotechnology. 7: 3535-3539.

b) For symposia reports and abstracts:

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (Pampus argentens euphrasen) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. Asian Fisheries Society Manila, Philippine 13: 191-199.

c) For edited symposia, special issues, etc., published in a journal:

Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science, 31: 17-27.

d) For books:

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

e) Books, containing sections written by different authors:

Kunev M (1979). Pig Fattening. In: A. Alexiev (Editor), Farm Animal Feeding. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg).

In referring to a personal communication the two words are followed by the year, e.g. (Brown, J. M., personal communication, 1982). In this case initials are given in the text.

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E.g. ANN: artificial neural network; CFS: closed form solution...

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Molar	mol/L	**Mililitre**	ml
Percent	%		

Other abbreviations and symbols should follow the recommendations on units, symbols and abbreviations: in "A guide for Biological and Medical Editors and Authors (The Royal Society of Medicine London 1977).

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6. In the English articles, a decimal point should be used instead of a decimal comma.
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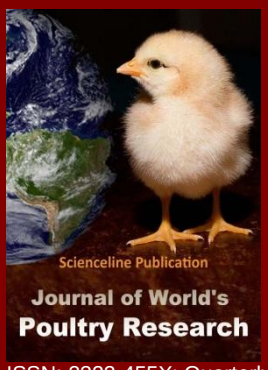

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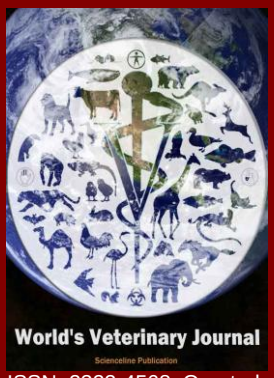

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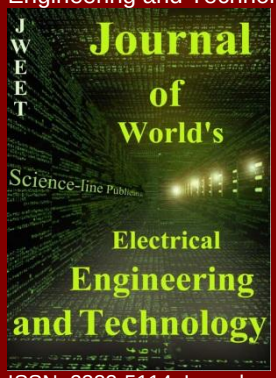

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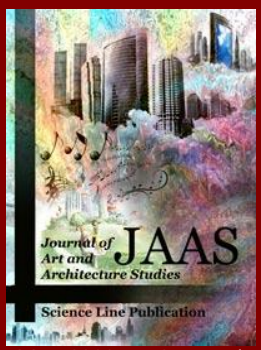

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