Research Article

Modulatory Effect of Dietary Allicin Supplementation on Productivity, Health, and Antioxidant Status of Ewes and their Offspring During May Breeding Season in Egypt

Doaa H. Assar[1], Rasha A. Al Wakeel[2], Mahmoud M. El-Maghraby[3], Mohammed M. El-Badawy[3], Adel A. El-Badawy[3], Wael M. Nagy[3], Mustafa S. Atta[2], Abdel-Khalek E. Abdel Khalek[4*]

[1]Department of Clinical Pathology, Faculty of Veterinary Medicine, Kafrelsheikh University, El-Geish Street, Kafrelsheikh 33516, Egypt; [2]Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, El-Geish Street, Kafrelsheikh 33516, Egypt; [3]Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt; [4]Department of Animal Production, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

Abstract | This study evaluated the dietary allicin supplementation on reproductive performance, milk production, blood parameters, and antioxidant status of ewes and the performance of their lambs during May breeding season. Crossbred ewes (n=48, 1/2 Finish Landrace x 1/2 Ossimi) with 2-5 y old at late pregnancy were distributed into four groups, 12 ewes in each. The controls were fed a control diet without supplementation. The other three groups were fed the same diet supplemented with allicin at the level of 0, 0.4, 0.8, and 1.2 g/kg, respectively. The experimental period lasted 150 days (45 d prepartum, 60 d suckling, and 45 d post-partum. Results showed that allicin (0.8 and 1.2 g/kg) had positive impacts (P<0.05) on ewe weight prepartum, and at lambing, suckling, and mating. Milk yield as 6% fat corrected milk, and fat and total solids per cent in milk increased (P<0.05) by allicin levels. Allicin (1.2 g/kg) improved (P<0.05) rates of estrus, pregnancy, lambing, and litter size. RBCs, hemoglobin, and neutrophils increased (P<0.05) by allicin (0.8 or 1.2 g/kg). WBCs increased (P<0.05) by allicin (1.2 g/kg), but lymphocytes and monocytes increased (P<0.5) by all levels. Allicin (0.8 and 1.2 g/kg) increased (P<0.05) total proteins and globulin, while decreased (P<0.05) creatinine, urea, total cholesterol, and triglycerides. Allicin supplementation reduced MDA and increased GSH, SOD, Gxp, catalase, and GPx. Allicin treatment of ewes (0.8 and 1.2 g/kg) increased (P<0.05) LBW and weight gain (total and daily) from birth to weaning, IgG, GSH, SOD, catalase, and GPx, while decreased MDA in blood serum of their lambs. Allicin dietary supplementation (1.2 g/kg) pre-May breeding season enhanced the productivity and general health of crossbred ewes and their lambs. Allicin exerts these positive impacts via its ability as a scavenger of free radicals by enhancing the endogenous antioxidant enzyme activities, metabolism, immunity, and reproduction.

Keywords | Allicin, Antioxidative enzymes, Healthy status, Immunity, milk, Lambing rat.

Received | October 24, 2022; **Accepted** | November 18, 2022; **Published** | December 20, 2022 ,
***Correspondence** | Abdel-Khalek E Abdel Khalek, Department of Animal Production, Faculty of Agriculture, Mansoura University, Mansoura, Egypt; **Email:** abdelkhalk2004@yahoo.com
Citation | Assar DH, Al Wakeel RA, El-Maghraby MM, El-Badawy MM, El-Badawy AA, Nagy WM, Atta MS, Khalek AKE (2023). Modulatory effect of dietary allicin supplementation on productivity, health, and antioxidant status of ewes and their offspring during may breeding season in egypt. Adv. Anim. Vet. Sci. 11(1): 83-93.
DOI | http://dx.doi.org/10.17582/journal.aavs/2023/11.1.83.93
ISSN (Online) | 2307-8316

INTRODUCTION

In Egypt, small ruminants are used as investments and insurance because of their high fertility, rapid generation times, and capacity for adaptation (Khalil et al., 2013). Exposing to different stresses from metabolism, environment, or nutrition causes oxidative stress, which alters a variety of biological activities and cellular activity (Ibrahim et al., 2012). Feed additives have been employed to increase animal output (Nassar et al., 2017). In this respect, antioxidants can help to protect function of the cells by increasing the antioxidant defense system's ability to counteract the oxidants' cellular alterations (Mittler et al., 2004). Several research revealed that chemical feed additives could have negative consequences and provide risks to both humans and animals (Schwarz et al., 2001). This could be a result of the hazardous residues in milk and meat, which pose a health risk (Russell and Houlihan, 2003). For this reason, the World Health Organization (WHO) advocates the use of natural feed additives to prevent the negative effects of artificial additives (Mohamed et al., 2003), such as dose-dependent, high manufacturing cost, level of toxicity, possible adverse side effects (digestive disorders, diarrhea, and nervous disorders) (Jha et al., 2020).

Phytobiotics gained a lot of attention in animal production because of their antioxidant, anti-microbial (Vamsi Duvvu et al., 2018), anti-inflammatory and immune-stimulatory (Moustafa et al., 2020) activities, and ability to lower lipid levels in the blood and accumulated lipid (Abdelnour et al., 2019; Latif et al., 2021). Garlic (*Allium sativum*) is one of the most important phytobiotics having a variety of medicinal purposes (Adulugba et al., 2017). Garlic's main compound, according to its phytochemical analysis is allicin (diallyl thiosulphate), a non-protein amino acid (Marchese et al., 2016), in addition to other allium compounds such as diallyl sulfide, γ-glutamyl-S-allyl-L-cysteines, and S-allyl-L-cysteine sulfoxides (alliin) and ajoene (Li et al., 2015; Suleria et al., 2015). Researchers discovered that garlic has a wide range of biological functions, including antimicrobial, anti-inflammatory, anti-atherosclerotic, anti-diabetic, anti-mutagenic, anti-carcinogenic, antioxidant, and immune-modulation properties (El-Sheakh et al., 2016; Singh et al., 2017). Phytochemicals have the ability to improve immunity and lipid profile (Abdelnour et al., 2019), and help in the regulation of glycolytic and gluconeogenic enzyme activity in the rat liver (Chetna et al., 2019).

Garlic extract has been used as a supplement in livestock production to boost growth performance (Ikyume et al., 2017), health status (Shokrollahi et al., 2016). Allicin, a biologically active substance extracted from garlic bulbs, can be chemically synthesized. Currently, the synthetic rate of chemical allicin is 85–90%, whereas the extraction rate from fresh garlic is only 0.3% (Nakamoto et al., 2020). The anti-inflammatory and immunomodulatory activities of garlic or its bioactive molecules and formulations were studied *in vitro and in vivo*. Allicin, as a garlic extract, appears to increase the functioning of the immune system via stimulation of some types of cells like macrophages, lymphocytes, natural killer cells, dendritic cells, and eosinophils, by modulating the cytokine secretion, production of immunoglobulins, phagocytosis, and activity of macrophages (Arreola et al., 2015). As a result of its low cost, high purity of active ingredients, and significant medicinal properties, chemically synthesized allicin has been widely used in animal production. (Chen et al., 2021). Recently, allicin was used as a strong natural antioxidant for improving the activity of antioxidant enzymes, decreasing the inflammation and oxidative stress in rabbits (Alam et al., 2018) and preventing apoptosis via activating extracellular signal-regulated kinase in rats (Abdel-Daim et al., 2017). Generally, allicin enhanced the reproductive performance, ovulatory response (ovulation rate, embryo quality, protein metabolism, antioxidant enzyme activities, and liver and kidney functions of doe rabbits (El-Ratel et al., 2020). Nevertheless, reported findings had no further support to the hypothesis of the allicin effects on the reproductive efficiency and healthy ewes during May breeding season in Egypt, which is characterized by low fertility of ewes in comparison with the September breeding season.

Therefore, the current study aimed to evaluate the role of dietary allicin supplementation, as feed additives, to improve growth, hemato-biochemical parameters, milk yield, immunity, and antioxidant status of crossbred ewes in May season. Also, the growth performance, immunity, and antioxidant status of their offspring were evaluated.

MATERIALS AND METHODS

The present study was carried out at Sakha Animal Production Research Station, Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt, in cooperation with the Faculty of Veterinary Medicine Kafrelsheikh University, Egypt. The experimental work was conducted during the period from January to Jun.

Animals, feeding system, and experimental design

A total of 48 pregnant ewes (Crossbred: 1/2 Finish Landrace x 1/2 Ossimi) with an age of 2-5 years and an average body weight of 45.51±1.49 kg was used in this study. All ewes were pregnant during the previous September breeding season, 2020. Ewes were kept in separate groups under semi-open sheds with the same managerial conditions. All ewes appeared healthy, sheared at the previous breeding season in September 2020 and free from any ailments.

The experimental ewes were fed on a diet including concentrate feed mixture (CFM) and fresh berseem (*Trifolium alexandrinum*, 2-3 cuts) with a roughage: concentrate ratio of 40:60, while fresh water and mineral salts were freely available throughout the experimental period. The diet was designed to meet the nutritional needs of ewes during late pregnancy and early postpartum according to NRC (2007). Chemical analysis on dry matter basis of CFM and fresh berseem according to the methods of the AOAC (1995) is shown in Table 1. The amounts of feed were adjusted according to the physiological and productive stages of ewes. The animals were weighed every two weeks and feed was changed according to variations in body weight and the animals' physiological conditions.

Ewes used as experimental animals in this study were pregnant during September breeding season and at late pregnancy at the beginning of the experimental period (January). The experimental ewes (n=48) were distributed into four groups, 12 ewes in each, according to live body weight and reproductive stage. Ewes in the control group were fed the diet (CFM and fresh berseem) without any supplementation. The other three groups were fed the same diet supplemented with allicin (Anhui Ruisen Biological Technology) at levels of 0.4, 0.8, and 1.2 g/kg (on DM basis), respectively. The trial lasted 150 days, including 40-45 d prepartum, 60 d suckling, and 40-45 d post-partum.

Experimental procedures

Live weight (LBW) of ewes was recorded 45- and 7-day prepartum, and at lambing, weaning, and mating. LBW of lambs was recorded at birth and weaning, and then total and average daily gain was computed during the birth-weaning interval. Milk production was estimated once/week for each ewe by a suckled-hand method according to Baker et al. (2009) from the 2nd to the 8th week of the suckling period. Amount of the suckled milk = difference between LBW of lambs separated from their dams for 12 h and suckling for 10 min. Daily milk yield = (the suckled milk + milk of hand milking after the suckling) x2. Fat corrected milk (FCM) yield can be calculated by the following equation:

FCM 6% (kg/d) = milk yield (kg/d) × [0.472+0.0088×fat (g/kg)] (Milis., 2008)

Samples of the milk were taken for chemical analysis for the percentages of fat, protein, lactose, solids not fat (SNF), and total solids (TS) by Milko-Scan (133 BN. FOSS Electric, Denmark).

Blood samples

Two blood samples (1st as whole blood and 2nd as serum) were collected from the jugular vein of 7 ewes and 5 lambs per group. Blood samples of ewes were taken at the end of the experimental period for hematological in the whole blood, and for biochemical parameters, lipid peroxidation markers, and antioxidant enzymes in blood serum. Blood samples of lambs were taken on the 3rd day of age for immunoglobulin, lipid peroxidation markers, and antioxidant enzymes in blood serum.

The first one was collected into a container containing EDTA to assess hematological parameters, including red blood cells (RBCs), hemoglobin (Hb), packed cells volume (PCV), white blood cells (WBCs), neutrophils, lymphocytes, monocytes, and eosinophils counts by the methods as described by (Coles, 1986; Coffin, 1995). The 2nd blood samples were collected without anticoagulant and left to clot, then centrifuged at 3000 rpm for 10 minutes to separate serum which was stored at -20 °c until used for analytical procedures.

Analytical procedures

Serum samples were analyzed for concentrations of total proteins and albumin (Henry et al., 1974), urea and creatinine (Siest et al., 1981), and triglycerides and total cholesterol (Richmond, 1973). However, globulin (Glob) concentration was computed by subtracting albumin concentration from TP. The serum enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also assayed as described by Reitman and Frankel (1957). All biochemical analyses were performed using spectrophotometer commercial kits (Bio diagnostics, Cairo, Egypt). All chemicals used in this study were of analytical grade.

Serum malondialdehyde (MDA) level was used to assess lipid peroxidation according to Ohkawa et al. (1979). The activity of glutathione peroxidase (GPx), superoxide dismutase (SOD), reduced glutathione (GSH), and catalase was determined in the blood serum of ewes or lambs according to Paglia and Valentine (1967), Owens and Belcher (1965), Beutler et al. (1963), and Aebi (1984), respectively. We bought all of our kits from Biodiagnostics, Cairo, Egypt.

Reproductive performance measurements

After a one-month postpartum period, estrus was detected by exposure ewes to ram teaser. To recognize ewes in heat, the teaser ram was employed twice daily, from 6 to 8 am and 3 to 4 pm. Ewes in heat were offered to fertile ram. One mating was served for ewes discovered in heat and another mating was served 12 h later. Ewes were deemed pregnant if they had gone through two estrous cycles without heating.

Estrus rate (number of ewes in heat/total number of ewes x100), conception rate (number of pregnant ewes/number of mated ewes x100), pregnancy rate (number of pregnant ewes/total number of ewes x100), lambing rate (number of pregnant ewes/numbers of lambed ewes x100), and twinning rate (number of ewes with twining/number of lambed ewes x100) was computed in each group.

Statistical analysis

The results were tabulated as the mean ± standard errors. Before analysis, data were tested for normality and homogeneity by Shapiro-Wilk's test and Levene's test, respectively. One-way analysis of variance (ANOVA) was used for all statistical analyses. A Chi-square test was used to analyze conception, pregnancy, lambing, and twining rates. The significant differences among means were separated by Duncan Multiple Range test and the significant differences were at values of $P<0.05$.

RESULTS AND DISCUSSION

Live body weight of ewes

Allicin supplementation at levels of 0.8 and 1.2 g/kg had a substantial positive impact ($P<0.05$) on ewe weight at late pregnancy (45 and 7-day pre-partum), lambing, weaning, and mating as compared to control. However, allicin treatment at a level of 0.4 g/kg increased ($P<0.05$) LBW of ewes only on Day 7 pre-partum and at weaning when compared to the control. Ewes receiving allicin at a level of 0.8/kg were the heaviest at all stages (Figure 1).

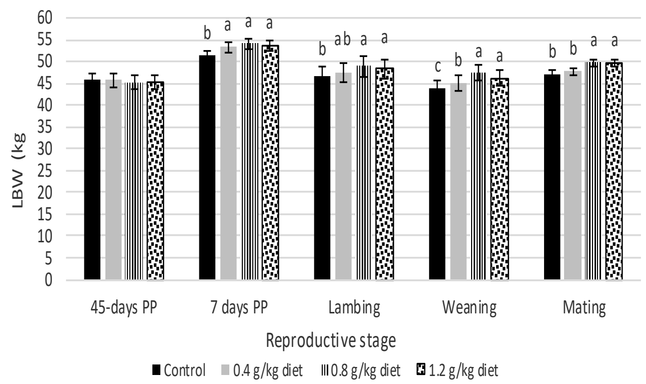

Figure 1: Average live body weight of ewes at late pregnancy (45- and 7-day pre-partum), lambing, weaning, and mating as affected by allicin administration. (a and b: Significant group differences at $P<0.05$)

These results indicated a beneficial effect of allicin treatment on LBW of ewes pre- and post-partum. The improvement observed in LBW of ewes may be attributed to that, allicin improves the rumen feed digestibility, which probably was at the expense of the flow of by-pass proteins to the small intestine in dairy cows (Ghosh et al., 2010). The dietary garlic oil administration decreased the concentration of reticular total short-chain fatty acids and tended to reduce the ruminal acetate-to-propionate ratio without effect on cattle ruminal pH value (Castillo-Lopez et al., 2021). Also, the addition of garlic juice in vitro was found to increase the production of propionate and decline the acetate-to-propionate ratio (Kekana et al., 2021). It is well recognized that antioxidants from natural sources are essential for enhancing the performance, immune system, and general health of the animal (El-Ratel et al., 2017). Antioxidants include anti-microbial, anti-inflammation, and immune-stimulating properties (Indrasanti et al., 2017), which may include in animal growth enhancement. It is of interest to note that the loss in LBW of ewes at lambing was lower in allicin treatment groups than in the control group. In this respect, Shams Al-dain and Jarjeis (2015) observed the impact of antioxidants (ginger) by losing lower weight in treated than in control cows.

Milk production

The average daily yield as a 6% fat-corrected milk and chemical composition of milk including fat and total solids percent were increased ($P<0.05$) by all allicin levels as compared to the control. The effect of allicin on milk yield and fat content was in a dose-response manner. However, allicin had a non-significant effect on percentages of milk protein, lactose, and solid not fat in treatment groups as compared to the control group (Table 2).

In association with enhancement in LBW of ewes in treatment groups, milk yield and contents of fat and total solids were improved ($P<0.05$, Table 2). According to several authors, animals treated with natural antioxidants such as black seed or ginger increased milk yield in ewes (El-ghousein, 2010) and dairy cows (Shams Al-dain and Jarjeis, 2015). In this context, the dietary inclusion of ginger powder (5 g/h/d) boosted the fat percentage in ewe milk (Hendawy et al., 2019). These impacts may be a result of the high efficiency of ruminal activity, which improved milk quality due to the general improvement of farm animals' immune systems. The increase observed in fat content in the milk of treatment groups may be caused by the altered ruminal acetate proportion by allicin administration (Kholif et al., 2012).

Reproductive performance of ewes in May breeding season

Allicin administration at a level of 1.2 g/kg improved ($P<0.05$) the reproductive performance of ewes in terms of increasing estrus rate, pregnancy rate, lambing rate, and litter size in comparison with control. However, allicin at a level of 0.8 g/kg improved ($P<0.05$) only pregnancy and lambing rates as compared to the control. On the other hand, the lowest level of allicin failed to change any of the

Table 1: Chemical composition of concentrate mixture (CM).

Item	Chemical composition (%)							TDN%
	DM	OM	CP	CF	EE	Ash	NFE	
Concentrate Feed Mixture*	92.33	92.41	14.36	11.08	3.16	7.59	63.81	78.76
Fresh berseem	17.25	88.13	14.08	25.95	1.88	11.87	46.22	74.52

Table 2: Effects of allicin treatment on milk yield and composition of ewes during the suckling period (60 days).

Item	Control group	Allicin group			*p*-Value
		0.4 g/kg diet	0.8 g/kg diet	1.2 g/kg diet	
6% fat corrected milk (g/d)	532.9±0.07^{c}	563.7±0.09^{b}	625.8±0.15^{a}	641.5±0.17^{a}	0.01
Milk composition					
Fat (%)	6.41±0.03^{c}	6.52±0.03^{b}	6.67±0.05^{a}	6.71±0.07^{a}	0.03
Protein (%)	5.42±0.13	5.63±0.17	5.61±0.17	5.58±0.13	0.13
Lactose (%)	5.33±0.41	5.26±0.40	5.18±0.38	5.12±0.38	0.41
Total solids (%)	17.91±0.52^{c}	18.12±0.55^{b}	18.22±0.52^{a}	18.17±0.52^{a}	0.05
Solids not fat (%)	11.50±0.47	11.60±0.47	11.55±0.42	11.46±0.42	0.47

$^{a, b, and c}$: Mean values within the same row with different superscripts are significantly different (P<0.05).

Table 3: Effects of allicin treatment on reproductive performance of ewes during May mating season.

Item	Control group	Allicin group		
		0.4 g/kg diet	0.8 g/kg diet	1.2 g/kg diet
Total number of ewes	12	12	12	12
Body weight at mating (kg)	47.2±0.81^{b}	47.8±0.84^{b}	49.8±0.80^{a}	49.8±0.85^{a}
Number of ewes with estrus	9	9	10	11
Estrus (mating) rate	9/12 (75.00^{b})	9/12 (75.00^{b})	10/12 (83.33^{ab})	11/12 (91.67^{a})
Number of pregnant ewes	8	9	10	10
Conception rate	8/9 (88.89)	9/9 (100)	10/10 (100)	10/11 (90.90)
Pregnancy rate	8/12 (66.66^{b})	9/12 (75.00^{ab})	10/12 (83.33^{a})	10/12 (83.33^{a})
Number of lambed ewes	7	8	9	10
Lambing rate	7/8 (87.50^{b})	8/9 (88.88^{b})	9/10 (90.00^{a})	10/10 (100^{a})
Total number of lambs born	9	10	12	14
Litter size (Prolificacy)	1.28±0.08^{b}	1.25±0.08^{b}	1.33±0.09^{ab}	1.40±0.09^{a}
Number of twins	2	2	3	4
Twining rate	2/7 (28.50^{b})	2/8 (25.00^{b})	3/9 (33.33^{ab})	4/10 (40.00^{a})

Note: Means within the same row with different superscripts are significantly different (P<0.05).
Estrus, conception, pregnancy, lambing, and twining rates were statistically analyzed by the Chi-square test.

reproductive performance parameters studied (Table 3).

The obtained beneficial effects on most reproductive performance parameters of ewes in May breeding season in the group that received the highest level (1.2 g/kg) as compared to the control or other allicin groups are similar to outcomes noted in ewes-fed diets with garlic in powder or oil form (Nassar et al., 2017) and in female rats administrated with garlic as aqueous extract (Raj et al., 2012). These results confirmed that ovulation and ovarian functions in rabbits were improved by natural antioxidants produced by plants (Zhong and Zhou, 2010). Also, dietary allicin supplementation was reported to be positive on the reproduction of chickens (El-Katcha et al., 2016).

In reproductive aspects, allicin administration showed a significant increase in ovulation rate, CLs number, and live litter size at birth in rabbits (El-Ratel et al., 2020) and in rats (Al-Masri, 2015). Allicin's numerous benefits for maintaining good health, including its anti-microbial, anti-inflammatory, and immunomodulatory qualities were reported (Indrasanti et al., 2017). Oral allicin administration significantly improved serum P4 levels during pregnancy and post-partum, and embryo quality in rabbits as compared to the control (El-Ratel et al., 2020). The increasing litter size and twining rate in ewes receiving allicin at a level of 1.2 g/kg diet may be attributed to that, high allicin level may increase GnRH (LH-RH) level, leading to an increase in LH level to keep CLs (Behnaz, 2014). Moreover, allicin treatment may improve embryo quality via a direct impact on ovarian tissues and/or an indirect impact on the health and immunity of ewes in treatment groups (El-Ratel et al., 2020).

Hematological parameters

Red blood cell (RBCs) count, hemoglobin (Hb) concentration, and neutrophils count increased (P<0.05 by allicin at a level of 0.8 or 1.2 g/kg. The count of WBCs increased (P<0.05) only by allicin (1.2 g/kg), but lymphocyte and monocyte count increased (P<0.5) by all allicin levels. On the other hand, PCV, erythrocytic values (MCV, MCH, and MCHC), and eosinophils were not affected by allicin treatment (Table 4).

Following the present results, several authors found an impact of natural antioxidant treatment on RBCs in Zaraibi kids treated with *Nigella sativa* (Habeeb and El-Tarabany, 2012). Also, RBCs, hemoglobin, PCV, platelets, and WBCs were improved in dairy cows fed ginger powder as compared to the control (Shams Al-dain and Jarjeis, 2015). The positive impact of medicinal plants on the hematological parameters may be attributed to folic acid, iron, and vitamin C as blood-forming factors that stimulate blood production in the bone marrow (Khattab et al., 2011).

Blood biochemicals

The present results indicated that allicin levels (0.8 and 1.2 g/kg) increased (P<0.05) concentration of total proteins and globulin concentrations, while decreased (P<0.05) creatinine, urea, total cholesterol, and triglycerides concentrations in blood serum of ewes. However, allicin at a level of 0.4 g/kg only decreased (P<0.05) serum total cholesterol and triglycerides. On the other hand, a non-significant effect was found for allicin treatment on the concentration of albumin and activity AST and ALT (Table 5).

Blood biochemical parameters can offer important details about the physiological status and give a sign of how healthy breeding ewes are. In our study, we observed an improvement in protein metabolism, in terms of increasing serum total proteins and globulin concentrations in ewes as affected by allicin treatment (0.8 or 1.2 g/kg). In this context, El-Ratel et al. (2020) reported that allicin treatment (10 mg/kg body weight) significantly enhanced serum total proteins, albumin, and globulin in doe rabbits, but serum AST and ALT activities were significantly decreased as compared to the control. We also found a slight reduction in transaminases activity (AST and ALT, P>0.05) in serum of ewes, as a marker of normality in liver function, whereas allicin also kept intact hepatic structure through the non-significant changes in serum hepatic injury biomarkers. Positive outcomes of allicin on liver function were reported on broiler chickens (El-Katcha et al., 2016) and rabbits (Alagawany et al., 2016) treated with allicin, and also in buffalo calves given garlic (Vamsi Duvvu et al., 2018). Moreover, aqueous garlic extract also helped rabbits with hepatic steatosis (Arhan et al., 2009). Organo-sulfur compounds found in garlic have a beneficial effect on liver function in rats (Ajayi et al., 2009) by maintaining cell membrane stability and shielding the liver from noxious substances and free radical-mediated toxic damage (Alam et al., 2018).

As markers of normal kidney function of ewes treated with allicin, urea, and creatinine levels were decreased (P<0.05) by allicin administration at levels of 0.8 and 1.2 g/kg diet. In agreement with this finding, Nassar et al. (2017) noted a decrease in urea concentration as a result of the inhibitory effect of garlic oil on deamination in ewes. Also, allicin had a positive effect on lipid profile by decreasing the level of total cholesterol and triglycerides of ewes in our study. Amin et al. (2015) stated that the periodic administration of five ml garlic extract in grazing lamb declined serum cholesterol concentrations. This can aid in lowering the accumulation of fat in mutton and enhancing the quality of the meat. This result indicated the hypo-lipidemic effect of allicin.

Lipid peroxidation and antioxidant enzyme activity

Allicin supplementation improved (P<0.05) lipid peroxidation by decreasing MDA level and increasing the activity of the endogenous antioxidant enzymes such as GSH, SOD, catalase, and GPx in comparison with the control. Allicin treatment at a level of 1.2 g/kg showed the highest impact on lipid peroxidation and antioxidant status of ewes (Table 6).

These results indicated that allicin, as an organic disulfide formed from alliin, exhibited potent antioxidant and anti-inflammatory actions (Abdel-Daim et al., 2019), capable of decreasing ROS signaling pathways and boosting the activity of endogenous antioxidant enzymes (Abushouk et al., 2017). Additionally, allicin may exert its effects directly or indirectly by activating phase II detoxifying enzymes through a nuclear factor erythroid 2-dependent route (Abdel-Daim et al., 2019). Although the SOD is crucial for shielding cells from ROS-related harm, this may be because the right nutrients are being added to the diet as supplements. (Ashour et al., 2014). In the same way, allicin treatment reduced the level of TBARS, as a lipid peroxidation marker, but did not affect the activity of GSH and SOD in doe rabbits (El-Ratel et al., 2020).

Growth performance, immunity, and antioxidant status of ewe offspring

Growth performance parameters of lambs produced by ewes treated with allicin at levels of 0.8 and 1.2 g/kg were increased (P<0.05) in terms of increasing LBW at birth and weaning as well as total and daily weight gain from birth to weaning in comparison with those produced from ewes in the control group and ewes treated with allicin at a level of 0.4 g/kg (Table 7).

Table 4: Effects of allicin treatment on hematological parameters of ewes at the end of the experimental period.

Item	Control group	Allicin group			*p*-Value
		0.4 g/kg diet	**0.8 g/kg diet**	**1.2 g/kg diet**	
RBCs (x10^6/µl)	10.7±0.45^b	10.94±0.41^{ab}	11.06±0.82^a	11.36±1.36^a	0.03
WBCs (x10^3/µl)	9.52±0.42^b	10.55±1.98^{ab}	10.65±0.5^{ab}	10.86±1.20^a	0.05
Hemoglobin (g/dl)	10.56±0.26^b	11.03±1.23^{ab}	11.50±0.50^a	11.73±1.23^a	0. 05
Packed cell volume (%)	30.1±2.66	32.33±2.4	31.58±2.72	30.9±0.57	0. 07
Erythrocytic values					
MCV (fl)	28.10±0.4	28.27±1.01	28.63±1.39	28.50±1.55	0.98
MCH (pg)	9.87±0.57	10.10±0.55	10.40±0.82	10.32±0.53	0.98
MCHC (%)	35.13±1.61	35.66±1.09	36.32±4.6	36.28±0.39	0.98
WBCs fractionation					
Neutrophils (x10^3/µl)	2.11±0.56^b	2.33±0.38^{ab}	2.48±0.41^a	2.46±0.18^a	0.05
Lymphocytes (x10^3/µl)	6.30±0.30^b	6.97±1.14^a	6.92±0.16^a	6.99±1.13^a	0.01
Monocytes(x10^3/µl)	0.82±0.06^b	0.95±0.05^a	0.94±0.07^a	1.09±0.09^a	0.05
Eosinophils(x10^3/µl)	0.29±0.10	0.30±0.23	0.31±0.11	0.32±0.04	0.76

Note: Means within the same row with different superscripts are significantly different (P<0.05).

Abbreviations: RBCs, red blood cells; WBCs, white blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 5: Effects of allicin treatment on biochemical analyses and activity of AST and ALT in serum of ewes at the end of the experimental period.

Item	Control group	Allicin group			*p*-Value
		0.4 g/kg diet	**0.8 g/kg diet**	**1.2 g/kg diet**	
Total proteins (g/dl)	6.56±0.13^b	6.58±0.13^b	6.73±0.14^a	6.85±0.15^a	0.03
Albumin (g/dl)	3.54±0.16	3.56±0.16	3.52±0.16	3.59±0.16	0.16
Globulin (g/dl)	3.02±0.13^b	3.02±0.13^b	3.21±0.14^a	3.26±0.14^a	0.04
AST (U/ml)	40.15±0.43	39.85±0.40	39.16±0.35	38.11±0.36	0.55
ALT (U/ml)	16.35±0.21	14.85±0. 20	14.75±0. 21	14.58±0.21	0.52
Creatinine (mg/dl)	1.13±0.03^a	1.14±0.03^a	0.95±0.13^b	0.93±0.13^b	0.04
Urea (mg/dl)	25.56±0.11^a	24.12±0.13^a	21.95±0.13^b	20.11±0.13^b	0.05
Total cholesterol (mg/dl)	156.6±1.12^a	131.7±1.13^b	120.3±1.02^c	114.6±1.02^c	0.03
Triglycerides (mg/dl)	111.80±0.96^a	99.81±0.96^b	92.35±0.96^b	87.56±0.96^c	0.01

Note: Means within the same row with different superscripts are significantly different (P<0.05).

Table 6: Effects of allicin treatment on lipid peroxidation markers and antioxidant enzymes in serum of ewes at the end of the experimental period.

Item	Control group	Allicin group			*p*-Value
		0.4 g/kg diet	**0.8 g/kg diet**	**1.2 g/kg diet**	
MDA (nmol/ml)	18.32±1.02^a	16.94±1.09^b	16.83±0.42^b	15.41±0.85^c	0.05
GSH (nmol/ml)	10.03±0.69^c	10.76±0.70^b	10.86±1.40^b	11.20±1.49^a	0.05
SOD (U/ml)	9.66±0.32^c	10.07±0.49^b	10.94±0.56^b	12.16±0.87^a	0.03
CAT (U/ml)	10.26±0.17^b	10.31±0.56^b	11.06±0.74^a	11.81±0.5^a	0.02
GPx (pg/ml)	4.44±0.90^c	5.83±0.57^b	6.47± 0.53^a	6.31±0.53^a	0.01

Note: Means within the same row with different superscripts are significantly different (P<0.05).

Abbreviations: MDA, malonaldehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase.

Treatment of ewes with all allicin levels significantly improved immunity, reduced lipid peroxidation (MDA), and increased antioxidant enzymes (GSH, SOD, catalase, and GPx in the blood serum of their lambs (Table 8).

The obtained improvement in growth and milk performance, health status, protein metabolism, and antioxidant status of ewes treated with allicin (0.8 or 1.2 g/kg) during pre- and post-partum reflected in beneficial effects on

Table 7: Effects of ewe treatment with allicin on growth performance of their lambs during the suckling period

Item	Control group	Allicin group			*p*-Value
		0.4 g/kg diet	0.8 g/kg diet	1.2 g/kg diet	
LBW at birth (kg)	3.41±0.24[b]	3.38±0.23[b]	3.75±0.20[a]	3.58±0.24[a]	0.04
LBW at weaning (kg)	12.35±0.44[b]	12.25±0.41[b]	12.95±0.52[a]	13.28±0.46[a]	0. 05
Total weight gain (kg)	8.94±0.95[b]	8.87±0.95[b]	9.20±0.96[a]	9.70±0.96[a]	0.05
Average daily gain (g/day)	149.0±0.41[b]	147.8±0.43[b]	153.3±0.52[a]	161.6±0.56[a]	0.05

Note: Means within the same row with different superscripts are significantly different (P<0.05).

Table 8: Effects of ewe treatment with allicin on immunoglobulin (IgG), malondialdehyde, and antioxidant enzymes in serum of their lambs at one week of age.

Item	Control group	Allicin group			*p*-Value
		0.4 g/kg diet	0.8 g/kg diet	1.2 g/kg diet	
IgG (mg/ml)	2.56±0.47[b]	5.43±1.30[a]	5.36±0.65[a]	5.74±0.73[a]	0.01
MDA (nmol/ml)	13.86±0.55[a]	10.65±0.74[b]	10.22±0.29[b]	10.43±0.29[b]	0.01
GSH (nmol/ml)	4.38±0.71[c]	7.25±1.64[b]	7.75±0.58[b]	8.03±0.16[a]	0.01
SOD (U/ml)	6.46±0.57[c]	6.95±0.98[b]	7.39±0.62[a]	7.98±1.51[a]	0.01
CAT (U/ml)	10.08±1.0[c]	9.99±0.35[c]	10.84±1.47[b]	12.10±1.59[a]	0.05
GPx (pg/ml)	4.76±0.90[c]	5.54±1.12[b]	6.14±0.78[a]	6.48±0.51[a]	0.05

Note: Mean values within the same row with different superscripts are significantly different (P<0.05).
Abbreviations: IgG, immunoglobulin type G; MDA, malonaldehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase.

their offspring in a dose-response manner. Abu Bakr (2019) stated that the body condition score of the dams and the preceding nutritional state of the ewes are two examples of factors that can affect the birth weight of lambs at birth and at weaning. The viability rate of kits increased by the treatment of doe rabbits with allicin (El-Ratel et al., 2020). In comparison with lambs of the control ewes, lambs of allicin-treated ewes showed significantly higher serum immunoglobulin (IgG) levels. Allicin improved the immunity of young animals (Wang et al., 2011) by elevating immunoglobulins levels of types IgG and IgM (Alam et al., 2018), and increasing phagocytosis and stimulating lymphocyte proliferation (Wang et al., 2011). This might be attributed to the positive effect of medicinal plants on the immune response that is advantageous for the animal as a whole (Lavinia et al., 2009).

The majority of natural antioxidants play an important role in increasing the reproduction, immunity, and health of rabbits (El-Ratel et al., 2017). Allicin, as a natural antioxidant, has many anti-microbial, anti-inflammation, immunomodulatory, and hypoglycaemic properties (Indrasanti et al., 2017). Garlic contains a lot of flavonoids, which stimulate the humoral immune response directly or indirectly by promoting B-cell proliferation and differentiation with accelerating T-cell proliferation (Acamovic and Brooker, 2005). Garlic has anti-microbial and immune-modulating effects due to its various organo-sulphur compounds, particularly allicin (Salem and Salem, 2016). Furthermore, garlic is able to decrease the growth and colonization of many pathogenic and non-pathogenic bacteria in the gut, which leads to better use of feed and improved immune response in growing rabbits (Nozarian et al., 2011) through its inhibitory effect on a specific thiol-containing enzyme in microorganisms with stimulation of intracellular bacterial thiol content oxidation by thiosulfate compounds in garlic (Lee and Gao, 2012). Improving the immune response of lambs is associated with increasing the activity of antioxidant enzymes (GSH, SOD, catalase, and GPx) and decreasing MDA levels in blood serum. Allicin administration (10 gm per kg LBW) exhibited the highest immunity response and lipid peroxidation of doe rabbits, in terms of increasing serum immunoglobulins (IgG and IgM), as immunity markers (El-Ratel et al., 2020).

CONCLUSION

According to the results of the current study, allicin dietary supplementation at a level of 1.2 g/kg during May breeding season enhanced the productivity and general health of crossbred ewes and their lambs. Allicin exerts these positive impacts via its ability to scavenge free radicals and enhance endogenous antioxidant enzyme activities. Allicin also modulates organ functions, metabolism, immune response, and reproductive performance. Allicin administration could be utilized to improve reproductive efficiency, milk production and composition, and to achieve higher gain and boost farmers' net income.

CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHOR CONTRIBUTION

Substantial contributions to conception and design (Doaa H. Assar, Mahmoud M. El-Maghraby, Wael M. Nagy, Abdel-Khalek E. Abdel-Khalek); acquisition of data (Rasha A. Al wakeel, Mohammed M. El-Badawy, and Mustafa S. Atta); analysis and interpretation of data (Doaa H. Assar, Mahmoud M. El-Maghraby, Adel A. El-Badawy, Wael M. Nagy, and Abdel-Khalek E. Abdel-Khalek); statistical analyses (Rasha A. Al wakeel, Adel A. El-Badawy, Mustafa S. Atta, and Abdel-Khalek E. Abdel—Khalek), drafting of the manuscript (Abdel-Khalek E. Abdel-Khalek, Mohammed M. El-Badawy, Mahmoud M. El-Maghraby, Wael M. Nagy, and Mustafa S. Atta); critically revising the manuscript for important intellectual content (Doaa H. Assar, Mahmoud M. El-Maghraby and Abdel-Khalek E. Abdel-Khalek); and final approval of the manuscript for publication (all authors).

ANIMAL WELFARES STATEMENT

The experimental procedures were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

ORCID ID:

Doaa H. Assar:0000-0001-7718-3560
Mahmoud M.El-Maghraby: 0000-0002-1542-4012
Abdel-Khalek E. Abdel-Khalek: 0000-0002-7839-2353

REFERENCES

Abdel-Daim M. M., Abushouk A. I., Donia T., Alarifi S., Alkahtani S., Aleya L., Bungau S. G. (2019). The nephroprotective effects of allicin and ascorbic acid against cisplatin-induced toxicity in rats. Environ. Sci. Pollut. Res. Int., 26: 13502–13509. https://doi.org/10.1007/s11356-019-04780-4

Abdel-Daim M. M., Kilany O. E., Khalifa H. A., Ahmed A. A. M. (2017). Allicin ameliorates doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. Cancer Chemotherap. Pharmacol., 80: 745–753. https://doi.org/10.1007/s00280-017-3413-7

Abdelnour S. A., Sheiha A. M., Taha A. E., Swelum A. A., Alarifi S., Alkahtani S. Ismail I. E. (2019). Impacts of enriching growing rabbit diets with chlorella vulgaris microalgae on growth, blood variables, carcass traits, immunological and antioxidant indices. Animals (Basel)., 9: 788–798. https://doi.org/10.3390/ani91 00788

Abu Bakr S. (2019). Effect of adding ginger powder or ginger oil on productive performance of ewes during lactation period. Egyptian J. Nutrit. Feeds, 22 (1): 63-78.

Abushouk A. I., Ismail A., Salem A. M. A., Afifi A. M., Abdel-Daim M. M. (2017). Cardioprotective mechanisms of phytochemicals against doxorubicin-induced cardiotoxicity. Biomed. Pharmacother., 90, 935–946. https://doi.org/10.1016/j.biopha.2017.04.033

Acamovic T., Brooker J. D. (2005). Biochemistry of plant secondary metabolites and their effects in animals. Proceed. Nutrit. Societ., 64: 403–412. https://doi.org/10.1079/PNS20 05449

Adulugba I. A., Goselle O. N., Ajayi O. O., Tanko J. T. (2017). Development of a potent anti-coccidial drug: A phytosynthetic approach. American J. Phytomed. Clin. Therapeut., 1, 1–7.

Aebi H. (1984). Catalase *in vitro*. Methods Enzymol., 105:121-126.

Ajayi G. O., Adeniyi T. T., Babayemi D. O. (2009). Hepatoprotective and some haematological effects of *Allium sativum* and vitamin C in lead-exposed wistar rats. Int. J. Med. Med. Sci., 1: 64–67.

Alagawany M., Elwy A. A., Fayez M. R. (2016). Effect of dietary supplementation of garlic (*Allium sativum*) and turmeric (*Curcuma longa*) on growth performance, carcass traits, blood profile and oxidative status in growing rabbits. Ann. Anim. Sci., 16: 489–505. https://doi.org/10.1515/aoas-2015-0079

Alam, R. T. M., Fawzi, E. M., Alkhalf, M. I., Alansari, W. S., Lotfy, A., Abdel-Daim, M. A. (2018). Anti-inflammatory, immunomodulatory, and antioxidant activities of allicin, norfloxacin, or their combination against *Pasteurella multocida* infection in male New Zealand rabbits. Oxidat. Med. Cellul. Longev., 4: 1–10. https://doi.org/10.1155/2018/1780956

Al-Masri S. A. (2015). Effect of pumpkin oil and vitamin E on lead induced testicular toxicity in male rats. J. Anim. Plant Sci., 25: 72–77.

Amin A. B., Yousuf M. B., Kolo U. M., Ibrahim A. A., Muhammad A. I., Maigado A. I. (2015). Influence of periodic administration of garlic extract on blood parameters of Grazing Lambs. Biokemistri, 26 (4): 114–119.

AOAC (2005). Official Methods of Analysis. 21st ed. Vol. 222. Washington, DC: Association of Official Analytical Chemists.

Arhan M., Öztürk H., Turhan N., Aytac B., Güven M., Olcay E., Durak I. (2009). Hepatic oxidant/antioxidant status in cholesterol fed rabbits: Effects of garlic extract. Hepatol. Res., 39: 70–77. https://doi.org/10.1111/j.1872-034X.2008.00401.x

Arreola R. Quintero-Fabián S., Ivette López-Roa R., Flores-Gutiérrez E. O., Reyes-Grajeda J., Carrera-Quintanar L., Ortuño-Sahagún D. (2015). Immunomodulation and Anti-Inflammatory Effects of Garlic Compounds. J. Immunol. Res., 2015: 401630.

Ashour E. A., Alagawany M., Reda F. M., Abd El-Hack M. E. (2014). Effect of supplementation of *Yucca schidigera* to growing rabbit diets on growth performance, carcass characteristics, serum biochemistry and liver oxidative status. Asian J. Anim. Vet. Adv. 9: 732–742.

Baker I. A., Dosky K. N., Alkass J. E. (2009). Milk yield and composition of Karadi ewes with the special reference to the method of evacuation. J. Duhok Univ., 12 (1): 210-215.

Behnaz H. (2014). Effects of garlic (*Allium sativum L.*) hydroalcoholic extract on estrogen, progesterone and testosterone levels in rats exposed to cell phone radiation. Zahedan J. Res. Med. Sci., 16: 19–24.

Beutler E., Duron O., Kelly B.M. (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882–888.

Castillo-Lopez E., Rivera-Chacon R., Ricci S., Petri R. M., Zebeli Q. (2021). Short term screening of multiple phytogenic compounds for their potential to modulate chewing behavior, ruminal fermentation profile, and pH in cattle fed grain-rich diets. J. Dairy Sci., 104 (4):4271–4289.

Chen J., Wang F., Yin Y., Ma X. (2021). The nutritional applications of garlic (*Allium sativum*) as natural feed additives in animals. Peer J., 10;9: e11934. https://doi.org/10.7717/peerj.11934. PMID: 34434661; PMCID: PMC8362672.

Chetna M., Monowar A. K., Nazmin F., Babita S., Dinesh T., Mohammad W., Abbas A. M. (2019). Effects of citral on oxidative stress and hepatic key enzymes of glucose metabolism in streptozotocin/high-fat-diet induced diabetic dyslipidemic rats. Iranian J. Basic Med. Sci., 22, 49–57. https://doi.org/10.22038/ijbms.2018.26889.6574

Coffin D.L. (1995). Manual of Veterinary and Clinical Pathology. 3rd ed., Comst. Pub. Ass. Inc. Ithaca., New York.

Coles E. H. (1986). Veterinary Clinical Pathology. Fourth edition. W.B. Saunders Company. Philadelphia.

Duncan D. B. (1955). Multiple range and multiple F-test. Biometrics., 11: 1–42. https://doi.org/10.2307/3001478

El-Ghousein S. S. (2010). Effect of some medicinal plants as feed additives on lactating ewe performance, milk composition, lamb growth and relevant blood items. Egypt. J. Anim. Prod., 47, 37-49.

El-Katcha M. I., Soltan M. A., Sharaf M. M., Hasen A. (2016). Growth performance, immune response, blood serum parameters, nutrient digestibility and carcass traits of broiler chicken as affected by dietary supplementation of garlic extract (Allicin). Alexandria J. Vet. Sci., 49: 50–64. https://doi.org/10.5455/ajvs.219261

El-Ratel IT, Abdel-Khalek A-KE, Gabr SA, Hammad ME, El-Morsy HI. (2020). Influence of allicin administration on reproductive efficiency, immunity and lipid peroxidation of rabbit does under high ambient temperature. J. Anim. Physiol. Anim. Nutr., 104:539–548. https://doi.org/10.1111/jpn.13316

El-Ratel I. T., Abdel-Khalek E. A., El-Harairy M. A., Sara F. F., Lamiaa Y. E. (2017). Impact of green tea extract on reproductive performance, hematology, lipid metabolism and histogenesis of liver and kidney of rabbit does. *Asian* J. Anim. Vet. Adv., 12: 51–60. https://doi.org/10.3923/ajava.2017.51.60

El-Sheakh A. R., Ghoneim H. A., Suddek G. M., Ammar M. A. (2016). Attenuation of oxidative stress, inflammation, and endothelial dysfunction in hypercholesterolemic rabbits by allicin. Canadian J. Physiol. Pharmacol., 94: 216–224. https://doi.org/10.1139/cjpp-2015-0267

Ghosh S., Mehla R. K., Sirohi S. K., Roy B. (2010). The effect of dietary garlic supplementation on body weight gain, feed intake, feed conversion efficiency, faecal score, faecal coliform count and feeding cost in crossbred dairy calves. Trop. Anim. Health Prod., 42 (5):961–968.

Habeeb A. A. M., El-Tarabany A. A. (2012). Effect of *Nigella sativa* or curcumin on daily body weight gain, feed intake and some physiological functions in growing Zaraibi goats during hot summer season. J. Radiat. Res. Appl. Sci., 5: 60-78.

Hendawy A. O., Mansour, M. M., Nour El-Din A. N. M. (2019). Effects of medicinal plants on haematological indices, colostrum, and milk composition of ewes. J. Vet. Med. Anim. Sci., 2 (1): 1-5.

Henry R. J., Canmon D. C., Winkelman J. W. (1974). Principles and techniques, Harper and Row. Clin. Chem. 415. https://doi.org/10.4061/2011/686137

Ibrahim N. M., Eweis E. A., El-Beltagi H. S., Yasmin E. A. (2012). Effect of lead acetate toxicity on experimental male albino rat. Asian Pacific J. Trop. Biomed., 2: 41–46. https://doi.org/10.1016/S2221-1691(11)60187-1

Ikyume T. T., Sowande O. S., Dele P. A., Yusuf A. O., Monday S., Egunjobi O. K., Fatoba O. (2017). Effect of varying levels of garlic (*Allium sativum*) powder on growth, apparent nutrient digestibility, rumen ecology, blood profile and cost analysis of feeding West African Dwarf goats. Mal. J. Anim., 20: 61–74.

Indrasanti D., Indradji M., Hastuti S., Aprilliyani E., Rosyadi K. (2017). The administration of garlic extract on Eimeria stiedai oocysts and the hematological profile of the coccidia infected rabbits. *Media Peternakan*, *3*, 158–164. https://doi.org/10.5398

Jha R., Das R., Oak S., Mishra P. (2020). Probiotics (Direct-Fed Microbials) in Poultry Nutrition and Their Effects on Nutrient Utilization, Growth and Laying Performance, and Gut Health: A Systematic Review. Animals., 10: 1863. https://doi.org/10.3390/ani10101863

Kata F. S., Athbi A. M., Manwar E. Q., Al-Ashoor A., Abdel-Daim M. M., Aleya L. (2018). Therapeutic effect of the alkaloid extract of the cyanobacterium *Spirulina platensis* on the lipid profile of hypercholesterolemic male rabbits. Environ. Sci. Pollut. Res., 25: 19635–19642. https://doi.org/10.1007/s11356-018-2170-4

Kekana, M. R., Luseba, D., Muyu, M. C. (2021). Effects of garlic supplementation on in vitro nutrient digestibility, rumen fermentation and gas production. South African J. Anim. Sci., 51 (2): 271–279.

Khalil, M. A., Sammour H. B., El-Wardani M. A. (2013). Socio-economic and technical evaluation of sheep and goat farms in north west cost of Egypt; Egypt. J. Sheep Goat Sci., 8 (1): 29-42.

Khattab H. M., El-Basiony A. Z., Hamdy S. M., Marwan A. A. (2011). Immune response and productive performance of dairy buffaloes and their offspring supplemented with black seed oil. Iran J. Appl. Anim. Sci., 1: 227-234.

Kholif, S. M., Morsy, T. A., Abdo M. M., Matloup, O. H., Abu El-Ella, A. A. (2012). Effect of supplementing lactating goats rations with garlic, cinnamon or ginger oils on milk yield, milk composition and milk fatty acids profile. J. Life. Sci., 4 (1): 27-34.

Latif A. A. E., Assar D. H., Elkaw E. M. (2021). Protective role of Chlorella vulgaris with Thiamine against Paracetamol induced toxic effects on haematological, biochemical, oxidative stress parameters and histopathological changes in Wistar rats. Sci. Rep. 11: 3911. https://doi.org/10.1038/s41598-021-83316-8

Lavinia S., Gabi D., Drinceanu D., Stef D., Daniela M.; Julean C., Ramona T., Corcionivoschi N. (2009). The effect of medicinal plants and plant extracted oils on broiler duodenum morphology and immunological profile. Romanian Biotechnolog. Lett., 14: 4606-4614.

Lee J., Gao Y. (2012). Review of the application of garlic, *Allium* *sativum*, in Aquaculture. J. World Aquacult. Societ., 43: 447–458. https://doi.org/10.1111/j.1749-7345.2012.00581.x

Li W., Zhou H., Zhou M. Y, Hu X. P., Ou S. Y., Yan R. A., Liao X. J., Huang X. S., Fu L. (2015). Characterization of phenolic constituents inhibiting the formation of sulfur-containing volatiles produced during garlic processing. J. Agric. Food Chem. 63 (3):787–794.

Marchese A., Barbieri R., Sanches-Silva A., Daglia M., Nabavi S. F., Jafari N. J., Izadi M., Ajami M., Nabavi S. M. (2016): Antifungal and antibacterial activities of allicin: a review. Trend. Food Sci. Technol. 52:49–56.

Milis Ch. (2008). Prediction of the energy value of ewe milk at early lactation. Acta Agriculturae Scandinavica, Section A-Anim. Sci., 58, 191-195. https://doi.org/10.1080/09064700802524479

Mittler R., Vanderauwera S., Gollery M., van Breusegem F. (2004). Reactive oxygen gene network of plants. Trend. Plant Sci., 9: 490–498. https://doi.org/10.1016/j.tplants.2004.08.009

Mohamed A. H., El-Saidy B. E., El-Seidy I.A. (2003). Influence of some medicinal plants supplementation: 1- On digestibility, nutritive value, rumen fermentation and some blood biochemical parameters in sheep. Egypt. J. Nutr. Feeds., 6 (2): 139-150.

Moustafa Eman M., Dawood M. A.O., Assar Doaa H., Omar Amira A., Elbialy Zizy I., Farrag, Foad A., Shukry M., Zayed M. M. (2020). Modulatory effects of fenugreek seeds powder on the histopathology, oxidative status, and immune related gene expression in Nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Aquacult., 51 (5), 734589.https://doi.org/10.1016/j.aquaculture.2019.734589

Nakamoto M., Kunimura K., Suzuki J. I., Kodera Y. (2020). Antimicrobial properties of hydrophobic compounds in garlic: Allicin, vinyldithiin, ajoene and diallyl polysulfides (review). Experimen. Therapeut. Med. 19(2):1550–1553.

Nassar M., El Shereef A., Abo Bakr S. (2017). Influence of feeding garlic plant either as powder or oil on reproductive performance of ewes. GSC Biolog. Pharmaceut. Sci., 1: 59–61.

Nouzarian, R., Tabeidian, S. A., Toghyani, M., Ghalamkari, G., Toghyani, M. (2011). Effect of turmeric powder on performance, carcass traits, humoral immune responses, and serum metabolites in broiler chickens. J. Anim. Feed Sci., 20: 389–400.

NRC (2007). National Research Council. Nutrient Requirements of Small Ruminants, Sheep, Goats, Cervids, and New World Camelids; National Academy Press: Washington, DC, USA, 2007.

Ohkawa H., Ohishi N., Yagi K. (1979). Assay tor lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyt. Biochem., 95:351-358.

Owens C., Belcher R. (1965). A colorimetric micro-method for the determination of glutathione. Biochem. J., 94:705.

Paglia D. E., Valentine W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Med., 70 (1):158-69.

Raj, L. O., Fayemi O. E., Ameen S. A., Jagun A. T. (2012). The effect of aqueous extract of allium sativum (garlic) on some aspect of reproduction in the female Albino Rat (*Wistar Strain*). Global Vet., 8: 414–420.

Reitman S., Frankel S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56–63.

Richmond W. (1973). Enzymatic determination of cholesterol. Clin. Chem., 19:1350.

Russell J. B., Houlihan A. J. (2003). Ionophore resistance of ruminal bacteria and its potential impact on human health. Fems. Microbiol. Rev., 27:65-74.

Salem N.A., E.A. Salem (2016). Protective antioxidant efficiency of garlic against lead-induced renal and testicular toxicity in adult male rats. J. Heavy Metal. Toxic. Dis., 1: 3–15. https://doi.org/10.21767/2473-6457.100015

Schwarz S., Kehrenberg C., Walsh T. R. (2001). Use of antimicrobial agent. In: veterinary medicine and food Anim. production. Int. J. Antimicro. Agents., 17: 431-437.

Shams Al-dain Q. Z., Jarjeis E. A. (2015). Vital impact of using ginger roots powder as feed additive to the rations of local Friesian dairy cows and its effect on production and economic efficiency of milk and physiological of blood. Kufa J. Vet. Med. Sci., 6:154-165.

Shokrollahi B., Hesami S. M., Baneh H. (2016). The effect of Garlic extract on growth, haematology and cell-mediated immune response of newborn goat kids. J. Agric. Rural Dev. Trop. 2016, 117: 225–232.

Siest G., Henny J., Schiele F. (1981). Interpretion des examens de laboratories, karger Ed., P. 206.

Singh J., Sharma M., Singh N., Kaur P., Sethi A., Sikka S. (2017). Effect of sun dried whole bulb garlic powder on nutrient utilization, blood parameters, duodenum morphology and faecal microbial load in broiler chickens. Indian J. Anim. Sci., 87: 195–198.

Suleria H. A. R., Butt M. S., Khalid N., Sultan S., Raza A., Aleem M., Abbas M. (2015). Garlic (*Allium sativum*): diet-based therapy of 21st century-a review. Asian Pacific J. Trop. Dis. 5(4):271–278.

Vamsi Duvvu M., Ananda Rao K., Venkata Seshaiah C. H., Srinivas Kumar D. (2018). Effect of garlic supplementation on the blood biochemical profile of Murrah buffalo calves. Int. J. Curr. Microbiol. Appl. Sci. 7: 2973–2983.

Wang J. P., Yoo J. S., Jang H. D., Lee J. H., Cho J. H., Kim I. H. (2011). Effect of dietary fermented garlic by *Weissella koreensis* powder on growth performance, blood characteristics, and immune response of pigs challenged with Escherichia coli lipopolysaccharide. J. Anim. Sci., 89: 2123–2131. https://doi.org/10.2527/jas.2010-3186

Zhong R., Zhou D. (2010). Oxidative stress and role of natural plant derived antioxidants in animal reproduction. J. Integrat. Agricult., 12: 1826–1838. https://doi.org/10.1016/s2095-3119(13)60412-8