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Research Article

Productive Performance of Doe Rabbits to Dietary Supplementation of *Aspergillus awamori*

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Abstract | A total number of thirty five, 9-10 month old New-Zealand white multiparous does, with an average weight of 3.54kg ± 0.096 were used to evaluate the response to dietary supplementation with *Aspergillus awamori* (AA) on some performance aspects during pregnancy and lactation periods. Does were allocated to 5 experimental groups, 7 does of each. Feeding on tested diets started one month before first mating. Does were allowed to the following treatments; a normal control group fed a basal diet without AA (T1), a positive control group fed T1 diet plus 0.1% *Bacillus subtilis* and *Bacillus licheniformis* (T2; Pro.), then the 3rd, 4th and 5th were fed the basal diet supplemented with 0.05%, 0.10% and 0.15% AA, respectively and abbreviated as 0.05% AA (T3), 0.10% AA (T4) and 0.15% AA, respectively. The trail was ended after pregnant period and nursering kits till weaning. The results indicate that doe rabbits fed diets supplied with 0.15% AA recorded significantly higher bunnies' size and total weight at weaning compared to control. Both groups fed either 0.10% or 0.15% AA recorded significantly higher total milk yield (7.71%) during the whole lactation period (5 weeks) comparing to un-supplemented group. All tested groups recorded a gradual decrease in Malondialdehyde (MDA) level in blood plasma by increasing AA levels in diets. None of the groups record any significant differences in immunoglobulin (IgM and IgA). It could be concluded that adding *Aspergillus awamori* to does diets up to 0.15% enhanced their performance during lactation period, in term of increased their milk production and litter weight at weaning.

Keywords | *Aspergillus awamori* , Doe rabbits, Milk production, Productive performance, Weaning bunnies.

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INTRODUCTION

Since 2006, European Union banded the use of antibiotics in livestock production; it was a big challenge to find alternatives to these feed additives. One of the most effective substances for antibiotics replacers is using probiotics. This might potentially benefit the host animal by improving its intestinal. Probiotics that contain yeast, live bacteria or bacterial spores can also prevent enteric disease of the rabbits. Instead of growth promoters with antibiotics that kill some of the rabbit own gastrointestinal flora, probiotic promote gut colonization and stabilize eubiosis by competitive growth against harmful micro-organism reducing the intestinal pH with production of lactic acid and encouraging digestion by producing enzyme and vitamins. This function strengthen the animals own new specific immune defense (Fortun- LaMothe and Drouet-Viard, 2002). In this respect, Nicodemus et al. (2004) concluded that feeding lactating doe rabbits with diet supplied by Toyocerin® (1×10 [9] CFU/g *Bacillus toyonenesis*) resulted in reducing parturition interval, increase feed efficiency and numerical productivity. Moreover, Phuoc and Jamikorn (2017) illustrated that supplementation of *L. acidophilus* (1×10^7 CFU/g) alone or in combination with *B. subtilis* (0.5×10^6 CFU/g *B. subtilis* plus 0.5×10^7 CFU/g *L. acidophilus*) could enhance number of gut beneficial bacteria populations, nutrient digestibility, cecal fermentation, feed efficiency, and growth performance of growing rabbits.

Asperigillus awamori (AA) is a fungus that has long been used for food processing in Japan. The products processed by AA are considered generally recognized as safe by the U.S. Food and Drug Administration (Bigelis and Lasure, 1987). In this point of view, Saleh et al. (2017) reported that supplementing laying hen diets with 0.05% AA improved their egg production compared with control group. They related this improvement to the increase in the feed efficiency of laying hens and metabolisable energy from the diet. The AA possesses the ability to digest raw starches according to (Amsal et al., 1999) and to produce cellulase and xylanase, which are required for the digestion of soluble non-starch polysaccharides (Bhat and Hazlewood, 2001).

It has been demonstrated that carcass weight increased in broiler chickens fed a diet containing 0.05% *A. awamori* (Yamamoto et al., 2007). Similarly, (Saleh et al., 2011) found that body weight gain and breast muscle weight increased, while feed intake and abdominal fat weight decreased when feeding broilers on a diet containing 0.05% *A. awamori*.

Regarding to antioxidant effect of AA, the increase in breast muscle TBARS levels in broilers as induced by exposure to heat stress, was decreased by dietary supplementation of AA (El-Deep et al., 2014). In addition, feeding diets containing *A. awamori* increased the mRNA expressions of antioxidant enzymes (i.e., glutathione peroxidase, catalase and superoxide dismutase) as shown by Zeweil et al. (2016). These results suggest that *A. awamori* can act as an antioxidant when fed to broiler chickens. Recently, El-Deep et al. (2021) concluded that addition of 100-150 mg AA/kg diet improved growing rabbits live weight, gain and feed conversion ratio.

Therefore, the aim of the present study was to evaluate the impact of dietary supplementation of *Aspergillus awamori* at different levels vs. commercial probiotics on productive performance of doe NZW rabbits during gestation and lactating periods. Finally, to determine the best and beneficial level that would improve bunnies' performance at weaning.

MATERIALS AND METHODS

The experimental work was carried out at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt.

Additives Used in This Study

Bio-Plus ® 2B (Chr. Hansen A/S, Denmark) is a thermo-stable probiotics containing 1×10^{12} *Bacillus subtilis* and *Bacillus licheniformis* spores per gram in a 1:1 ratio.

Cultivation of *Aspergillus awamori* (AA) and its toxins analysis: *Aspergillus awamori* was cultivated using steamed rice at the Biogenkoji Research Institute (Kirishima, Japan). The final product was analyzed in Central Lab of Residue analysis of Pesticides and Heavy Metals in Food (QCAP lab), department of mycotoxins and food additives analysis in food, according to the following Figures (1, 2), Injection of main four types of aflatoxin (B1, B2, G1, and G2) standard on high-performance liquid chromatography with fluorescence detector has been verified in Figure (1), well-defined highly separated peaks have been observed.

The injected sample chromatogram presented in Figure 2, conceal a clear sample from any aflatoxin consequently it safe to use through the intended experiment

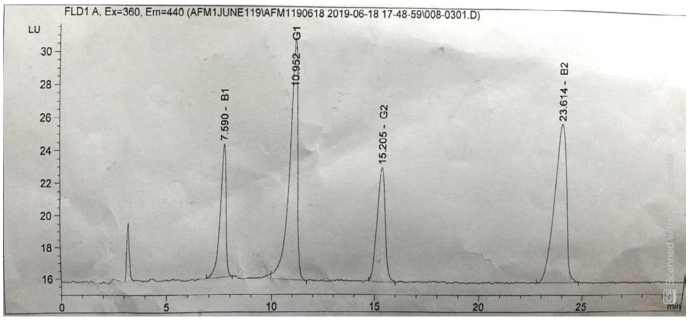

Figure 1: results of injecting four types of toxins (B1, B2, G1, G2)

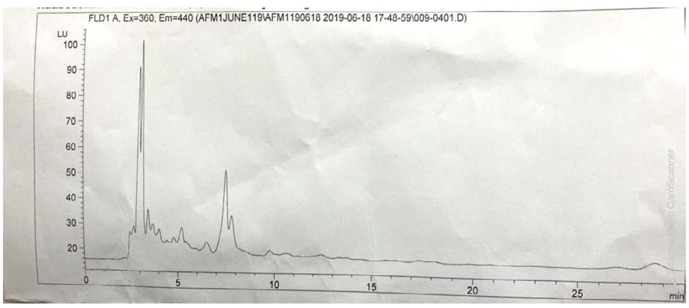

Figure 2: show a safe use of *Aspergillus awamori* for feeding animals

Table 1: Ingredients and calculated analysis of the basal diet

Ingredient	%	Calculated chemical analysis	
Barley	17.5	Crude protein (%)	18.26
Wheat bran	32.2	Digestible energy (kcal/kg)	2620
Soybean meal (44%)	13.6	Crude fiber (%)	11.14
Clover hay (12%)	17.9	Ether extract (%)	2.75
Corn gluten (60%)	3.5	Calcium (%)	1.23
Yellow corn	11.5	Total phosphorus (%)	0.81
Limestone	1.5	Lysine (%)	0.82
Di-Calcium phosphate	1.5	Methionine (%)	0.51
NaCl	0.3	Methionine + Cystein	0.84
Vitamins and minerals premix*	0.3	Sodium	0.16
Dl- Methionine	0.2		
Total	100		

*Each 3 kg contain: 6000000 IU Vit. A; 900000 IU Vit. D3; 40000 mg Vit. E; 2000 mg Vit. K3; 2000 mg Vit. B1; 4000 mg Vit. B2; 2000 mg Vit. B6; 10 mg Vit. B12; 50 mg Biotin; 10000 mg Pantothenic acid; 50000 Niacin; 3000 mg Folic acid; 250000 mg Choline; 8500 mg Mn; 50000 mg Zn; 50000 mg Fe; 200 mg I; 100 mg Se, 5000 mg Cu, and 100 mg Co.

Table 2: Effect of different treatments on doe rabbits performance during gestation period.

Treatments	Mother weight (Kg)	Feed intake (Kg)	Litter size at birth	Total weight at birth (g)	Weight (g)/bunny
T1 (Control)	3.70	3.49 ^{BC}	7.00	275.00	39.29
T2 (Pro)	3.59	4.02 ^{A}	7.00	280.00	40.00
T3 (0.05%AA)	3.54	3.37 ^{BC}	7.00	306.67	44.56
T4 (0.10% AA)	3.60	3.12 ^{C}	7.29	286.67	39.32
T5 (0.15% AA)	3.79	3.71 ^{AB}	7.43	300.00	40.38
SE	0.096	0.147	0.258	8.72	2.43
Sig.	NS	**	NS	NS	NS

**A, B and C Means in each column, with same superscripts are not significantly different (P≤0.01). AA: *Aspergillus awamori,* Pro: Probiotics, NS: not significant

Table 3: Effect of different treatments on doe rabbits performance during lactation period.

Treatments	Feed intake (Kg)	Litter size at weaning	Total weight at weaning (g)	Weight (g)/bunny
T1 (Control)	6.06	6.57 ^{b}	2670.71 ^{C}	408.27
T2 (Pro)	6.60	6.57 ^{b}	2759.29 ^{B}	422.11
T3 (0.05%AA)	6.27	7.00 ^{ab}	2777.86 ^{B}	398.64
T4 (0.10% AA)	6.62	7.14 ^{ab}	2791.43 ^{B}	391.43
T5 (0.15% AA)	6.48	7.29 ^{a}	2880.00 ^{A}	396.46
SE	0.203	0.191	18.94	9.436
Sig.	NS	*	**	NS

*a and b Means in each column, with same superscripts are not significantly different (P≤0.05).
**A, B and C Means in each column, with same superscripts are not significantly different (P≤0.01). AA: *Aspergillus awamori,* Pro: Probiotics, NS: not significant

Experimental Animals, Design and Management

Thirty five multi-parious New Zealand White does 9-10 months old weighing 3.54 kg ±0.096 were equally allocated to four groups as follows:

T1 (Control): fed basal diet without supplementation.

T2 (Control positive): fed basal diet + 0.1% commercial probiotics (Bio-Plus® 2B; Pro)

T3: fed basal diet + 0.05% *Aspergillus awamori* (0.05% AA).

T4: fed basal diet + 0.10% *Aspergillus awamori* (0.10% AA).

T5: fed basal diet + 0.15% *Aspergillus awamori* (0.15% AA).

Each group had 7 does, which were fed these diets for month before first mating. Detection of conception was carried out by palpation at 10 days after mating and the non pregnant were re-mated immediately. Does were housed in individual wired-cages (60×50×40 cm). All animals were kept under the same management and hygienic conditions and provided with fresh water and pelleted diets *ad-libtum* over the experimental period.

Experimental Diets and Measurements

Diets were formulated to meet the NRC (1977) requirements during pregnancy and lactation periods. Ingredients and calculated analyses of the basal diet are presented in Table 1. Variables of doe rabbit's weight, doe feed intake, litter size and weight at birth and at weaning were considered. Individual blood samples were collected from three does of each treatment from the marginal ear vein in 5 ml heparinized test tubes and centrifuged at 3000 r.p.m for 20 minutes then plasma were stored at -20°C until measurements of plasma antioxidants being Malondialdehyde (MDA; nmol/ml), Total antioxidant capacity (TAOC; mmol/l) according to Piette and Raymond (1999), also, determine some immune parameters such as IgA (mg/dl), IgM (mg/dl) and IgG (mg/dl) by using commercial kits.

Milk Production and Composition

Milk production (ml) was estimated by using doe-suckle-weigh method (Lukefahr et al., 1983). Milk samples were drawn on d 21 after parturition. Milk composition was evaluated for total lipid (mg/dl), total protein (g/dl), and albumin (g/dl) content by infrared analyzer with a Milkoscan (Milk-o-Scan 605, Foss Electric, Hillerd, Denmark) according to the method of El-Sayiad et al. (1994). While, globulin (g/dl) was calculated by subtracting albumin from total protein and A/G ratio was also calculated.

Statistical Analysis

The obtained data were statistically analyzed using one-way analysis of variance procedure (SAS, 2004) computer program using the following model:

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

Where Y_{ij} = the individual observation, μ = overall mean, T_i = effect of treatments (i = 1, 2, 3, 4 and 5). e_{ij} = random error.

Significant differences between treatments means were determined at (P≤0.05) and (P≤0.01) by Duncans multiple-range test (Duncan's, 1955).

RESULTS AND DISCUSSIONS

Effect of different treatments on performance of doe rabbits during gestation period is listed in Table (2), it is worthy to note that all parameters did not affected significantly by various treatments except feed intake values, which were significantly high (4.02 Kg) in group of T2 (Pro) and the lowest value was recorded for T4 (0.10 % AA). While, FI in the rest of all groups were located intermediate between them. The increase in FI in group fed probiotics like the same conclusion of Meeske et al. (2002) who documented that supplementation of lactic acid bacteria (Probiotics) to maize at ensiling enhanced the palatability and intake of silage compared to control. Also, Sakr (2017) found that adding *Aspergillus awamori* to growing rabbit diets contaminated with aflatoxin, increased FI during growth period.

Table (3) show that FI values and individual bunnies' weight didn't affected significantly during lactation period. The results came to the same line with Nicodemus et al. (2004) who concluded that addition of Toyocerin ® (containing *Bacillus cereus*) to doe rabbits, did not show any significant variations in their daily feed intake during lactation period. On the other hand, the group fed 0.15% AA (T5) recorded significantly the highest litter size at weaning with a 10.96% increament than both normal control (T1) and positive control (T2; Pro). Whereas, group of T4 (7.14) and T3 (7.00) recorded interval value between them. Regarding to total litter weight at weaning, group of T5 (0.15% AA) achieved significantly the highest value followed by T3 (0.05% AA), T4 (0.10 % AA), T5 (0.15%AA) and the lowest value was for T1 (normal control). The results are in accordance with the previous studies of Maertens et al. (1994) and Nguyen et al. (1988), in which litter weight was significantly improved (8% compared to control) by feeding diets supplemented with probiotics (Paciflor®), the enhancement of bunnies weight at weaning may be due to modification of host metabolism, stimulation of immune system, anti-inflammatory reactions, knock off and insularity of harmful bacteria in small intestine, which reflect on the enhancement of nutrients absorption. Hence improve growth performance (Edens, 2003). Also, Nicodemus et al. (2014) explained the enhancement of doe rabbits by feeding diets with Toyocerin® by the same conclusion. The improvement in weight gain and feed efficiency due to the use of AA may be partially due to the increase in metabolic energy of the feed as reported by Saleh et al. (2012a). In broilers, Gibson and Roberfroid (1995) related the enhancement of chicks' performance and welfare to activate the metabolism of one or a limited number of health-promoting bacteria or by selectively stimulating their growth. Lately, El-Deep et al. (2021) concluded that addition of *A. awamori* continuously for 8 weeks in growing rabbits enhanced their growth performance.

Results in Table (4) show that all tested groups recorded higher milk production during the first, third, fourth weeks as well as total milk production period (1 – 5 weeks). During the first week, the group of T4 (0.10% AA) recorded significantly the highest milk production rate with 17.54% and 7.19% more than normal control (T1) and positive

Table 4: Effect of different treatments on milk production.

Treatments	Week 1 (ml/doe)	Week 2 (ml/doe)	Week 3 (ml/doe)	Week 4 (ml/doe)	Week 5 (ml/doe)	Total milk production (ml/doe)
T1 (Control)	81.43 C	172.86	182.14 b	176.43 B	30.43	643.29 B
T2 (Pro)	89.29 B	165.71	194.29 a	188.57 A	30.71	668.57 AB
T3 (0.05%AA)	90.71 B	182.14	179.29 b	185.71 A	33.57	671.42 AB
T4 (0.10% AA)	95.71 A	182.86	194.29 a	187.86 A	32.14	692.86 A
T5 (0.15% AA)	91.43 AB	187.14	190.00 ab	190.71 A	33.57	692.85 A
SE	1.52	7.17	3.78	2.81	1.65	9.77
Sig.	**	NS	*	**	NS	**

*a and b Means in each column, with same superscripts are not significantly different (P≤0.05).
**A, B and C Means in each column, with same superscripts are not significantly different (P≤0.01). AA: *Aspergillus awamori,* Pro: Probiotics, NS: not significant

Table 5: Effect of different treatments on milk composition.

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Total lipids (mg/dl)
T1 (Control)	13.01 C	7.34 C	5.67 C	1.30 BC	1039.31A
T2 (Pro)	18.24 A	10.48 A	7.75 A	1.35BC	616.92 D
T3 (0.05%AA)	15.99 B	9.27 B	6.72 B	1.38 AB	955.56 B
T4 (0.10% AA)	17.93 A	9.81 AB	8.12 A	1.21 C	651.67 D
T5 (0.15% AA)	16.41 B	9.89 AB	6.52 B	1.52 A	719.49 C
SE	0.281	0.224	0.151	0.048	13.350
Sig.	**	**	**	**	**

**A, B, C and D Means in each column, with same superscripts are not significantly different (P≤0.01). AA: *Aspergillus awamori,* Pro: Probiotics, NS: not significant

Table 6: Effect of different treatments on doe rabbits blood plasma parameters.

Treatments	Blood antioxidant parameters		Immune parameters		
	MDA nmol/ml	TAOC mmol/l	IgA (mg/dl)	IgM (mg/dl)	IgG (mg/dl)
T1 (Control)	2.90 A	1.63 A	42.67 A	7.00	115.67
T2 (Pro)	2.38 B	1.54 B	34.33 C	6.67	114.33
T3 (0.05%AA)	1.23 C	1.68 A	37.67B	6.33	115.67
T4 (0.10% AA)	1.08 C	1.61 A	39.67 AB	7.67	114.00
T5 (0.15% AA)	1.07 C	1.65 A	40.33 AB	6.33	114.00
SE	0.109	0.021	0.988	0.554	3.85
Sig.	**	**	**	NS	NS

**A, B and C Means in each column, with same superscripts are not significantly different (P≤0.01). AA: *Aspergillus awamori*, Pro: Probiotics, NS: not significant

control (T2), respectively. While at the third week of lactation, both groups of T4 (0.10% AA) and T2 (Pro) achieved better milk production rate than T5 (0.15% AA) without significant differences, but the same groups recorded significant variations to T3 (0.05% AA) and normal control (T1). In this connection, Kim et al. (2001) observed higher total milk production of dairy cows treated with 0.5% probiotics than others fed control diet. Also, Sablik (2002) concluded that supplementing probiotics (*Saccharomyces cerevisiae*) to dairy cow diet at level of 15g/kg given higher milk yield compared to control group.

All groups recorded significantly higher milk rate than normal control (fourth week of lactation). During total milk production period, does fed the highest levels of AA (T4; 0.10% AA, T5; 0.15% AA) recorded an increment in milk yield by 7.71% than the normal control. Whereas, the other groups of T3 (0.05% AA), T2 (Pro) recorded intermediate value without significant differences between higher groups (T4 and T5) and the normal control. The previous aspects may be considered as explanations of the enhancement effect observed in all group in total weight at weaning as mentioned before in Table (3). The heightening in milk production during feeding both *Aspergillus awamori* and *Saccharomyces cerevisiae* could be due to increasing mRNA expression of glutathione peroxidase which support the finding of antioxidant properties of them and have a special role in maintaining animal health as well as productive and reproductive performance (Saleh et al., 2013) which reduce the physiological response to stress in animals, increase the protection of tissues (as mammary gland cells) against lipid peroxidation (Ohtsuka et al., 1998).

Milk composition as affected by different treatments is presented in Table (5), milk total protein and globulin were increased in groups of T4 (17.93 g/dl, 8.12 g/dl, respectively) and T2 (18.24 g/dl, 7.75 g/dl, respectively) followed by groups of T3 (15.99 g/dl, 6.72 g/dl, respectively) and T5 (16.41 g/dl, 6.52 g/dl, respectively) then finally the group of normal control (T1) being 13.01 g/dl and 5.67 g/dl, respectively. Milk albumin was high in group fed T2 diet while, rabbits fed T4 and T5 diets recorded lower albumin value than T2 group without significant differences between them. Whereas, group of T3 recorded intermediate value (9.27 g/dl) between the former groups and un-supplemented group (7.34g/dl). In this study, milk composition (globulin) was increased as a result of adding either commercial probiotics or *Aspergillus awamori*, which could reflected on the immune response of rabbits (ElKatcha et al., 2011). The increased total milk protein indicates that the protein content of the diets was adequate and available and that *A. awamori* inclusion levels increased the protein metabolism and synthesis through the activation of protease (Olorunsola et al., 2016). Furthermore, de Castro et al. (2015) related the improvement in protein absorption to the degradation of anti-trypsin and anti-nutritional factors in soybean meal and other ingredients from plant origin as a result of adding *Aspergillus*. In this respect, Iwanska et al. (2000) reported that feeding lactating cows with diets supplemented with probiotics achieved higher milk protein than others fed control diet.

Regarding to milk A/G ratio, it is worthy to note that the group of T5 achieved significantly the highest value (1.52) while, T4 group recorded the lowest one (1.21). total lipid in does milk was gradually decreased by supplementing them different feed additives, where the normal control (T1) recorded significantly the highest level (1039.31 mg/dl) followed by T3 (955.56 mg/dl) then T5 (719.49mg/dl) and finally both groups of T4 and T2 being 651.67 and 616.92 mg/dl, respectively. The lowering in milk total lipids as a result of enriching doe rabbit diets with AA may be related to the inhibition of 3-hydroxyglutaryl-coenzyme (HMG-CoA) reductase, which lowers cholesterol levels (Saleh et al., 2011), this enzyme (HMG-CoA reductase) inhibitor is excreted by either fungus, *Penicillum citrinum* (Endo, 1985) or *Aspergillus awamori* (Mersmann, 1998). It is safe to treat patients who suffer from hypercholesterolemia (Serruys et al., 2002). Also, *Aspergillus awamori* may be responsible for the activities of hormone-sensitive lipase and malate dehydrogenase enzyme in some tissues as reported by Mersmann (1998).

As shown in Table (6), Malondialdehyde (MDA) is one of the most frequently used indicators of lipid peroxidation. All tested groups fed different AA levels (T3, T4, T5) recorded a significant decrease in plasma MDA compared to normal (T1) and positive (T2) controls. On the other hand, rabbits fed (T2) diet recorded significantly the lowest TAOC compared to normal control (T1) or other groups fed various levels of AA (T3, T4, T5). This result agree with the previous research of Saleh et al. (2012b) who concluded that supplementing broilers diet with 0.05% AA resulted in reducing muscle TBARS and increasing n-tocopherol content which indicate that AA has an anti-oxidant properties, the same conclusion was previously documented by Kaminishi et al. (1999) who reported that some antioxidant materials were produced by *Aspergillus awamori*. The reduction in TBARS may be explained by substances which produced by *Aspergillus awamori*, hence by feeding diets enriched with antioxidant, the oxidative status of broilers was improved (Saleh et al., 2011). Also, Lee et al. (2007) and Bhanja et al. (2009) found an increament in total phenolic and anthocyanin contents during the fermentation process done by AA, which leads to increase the antioxidant activity. On the other hand Kanauchi et al. (2008) showed that a natural material called feruloyl esterase could contribute in enhancing the antioxidative proprieties of *Aspergillus awamori*.

Regarding to plasma IgA level, the group of T1 recorded the highest level (42.67mg/dl) followed by T4 (39.67 mg/dl) and T5 (40.33 mg/dl) without significant differences between them, then the group of T3 (37.67 mg/dl) and the lowest value (34.33 mg/dl) was recorded for T2. Whereas, none of the plasma IgM and IgG affected significantly by different treatment studied. In this respect, Sakr (2017) reported that addition of AA at different levels (0.05%, 0.10% and 0.15%) to growing rabbit diets improved their immunity in term of increasing phagocytic activity and phagocytic index.

CONCLUSION

It could be concluded that supplementing doe rabbit diets with *Aspergillus awamori* up to 0.15% enhanced their performance as an increase in milk production and its quality which reflected finally in improving bunnies' weight at weaning.

NOVELTY STATEMENT

The results declare that *Aspergillus awamori* is considered as probiotic and has antioxidant properties which affected positively the productive performance of doe rabbits.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors are contributed equally.

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