2024 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 14, Issue 1: 13-21; January 27, 2024

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2024.2

EFFECTS OF DIETARY SUBSTITUTION OF SOYBEAN MEAL BY DRIED AZOLLA ON BLOOD AND SERUM PARAMETERS, PRODUCTIVE AND REPRODUCTIVE TRAITS, AND ECONOMIC EFFICIENCY OF RABBIT DOES AS WELL AS SEMEN QUALITY OF BUCKS

Mohamed M. EL-DEEB[1]✉, Ahmed M. ALAZAB[2], Soheir A. SHAZLY[3], Hany N. FAHIM[2], and Mona A. RAGAB[2]

[1]*Animal Nutrition Research Department, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt*
[2]*Rabbit, Turkey and Water-Fowl Breeding Research Department, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt*
[3]*Poultry Research Department, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt*

✉Email: deep121eg@yahoo.com

Supporting Information

RESEARCH ARTICLE

PII: S2228770124000002-14

Received: September 11, 2023

Revised: January 06, 2024

Accepted: January 08, 2024

ABSTRACT: An experiment was carried out to investigate the effects of dietary substitution of soybean meal in different ratios by dried Azolla (*Azolla pinnata*) on productive and reproductive performances, hematological and serum traits, and economic efficiency of rabbit does as well as semen quality of males. Forty mature does and eight males of Black Balady rabbits aged seven and eight months were assigned to 1 of 4 dietary groups: 0 (control), 20, 30, and 40% of soybean protein substituted with dried Azolla protein. Data were analyzed using repeated measures of statistical software computer program package. There were no significant differences among groups in number of services per conception as well as parturition intervals (days) with a superiority of 30% group over the control and other two groups. Average litter weight was significantly ($P<0.05$) superior in the 30% replacement group. There were no significant differences in live body weight either at birth or at weaning among the four tested groups and the 30% replacement groups recorded the highest significant daily weight gain of bunnies during the whole experimental period. All Azolla groups recorded better results in the studied traits of bucks' semen compared to the control. Azolla diets did not show any adverse effects on the studied blood parameters. The 30% replacement of the soybean protein group showed the best economic return compared to the other two replacement groups and the control. In conclusion, Azolla can safely and economically replace soybean protein at the rate of 30% in adult female rabbits' diets.

Keywords: Azolla, Body weight, Rabbit, Reproduction, Semen.

INTRODUCTION

Nowadays, the animals' performance and lower feeding costs are major concerns by researchers all over the world due to the global economic crises. In the diets, nearly 75% of livestock operation costs are associated with feed costs (Issa and Abo Omar, 2012; Tubb and Seba, 2021). The shortage of fodder is therefore substituted with concentrate feed, increases animal production costs. Fasuyi and Aletor (2005) stated that green plants early considered the most probable potential source and cheapest protein due to their power in synthesizing amino acids from various available materials. The technology of green fodder production is especially important in some countries where forage production is limited (Abo Omar et al., 2012). So far reasonable cost potential feed quality is a key to successful livestock production projects.

The need for using alternatives to concentrate feeds led scientists to one valued plant namely Azolla. Azolla is a floating fern grown in shallow water belongs to the family *Azollaceae* (DeFrank, 1995). Azolla has a unique symbiotic relationship that makes it a perfect plant with high protein content (Pillai et al., 2001; Mohamed et al., 2018). This plant is considered a promise of providing a cheap source of protein in livestock feeds (Samad et al., 2020; Nasir et al., 2022). Chemical analysis has shown that Azolla is not only very rich in proteins, but also it has valuable essential amino acids content, vitamins (such as A, B12, Beta-carotene) and growth promoter substances and minerals, e.g. calcium, phosphorus, potassium, ferrous, copper, magnesium, etc., as reviewed and discussed by Roy et al. (2016). Azolla is also characterized by phytochemical properties that make it with diverse pharmacological influences, e.g. antioxidant, anti-carcinogenic, anti-allergic, and anti-inflammatory due to its content from flavonoids, phenolic, tannins, and saponins (Mithraja et al., 2011; Thangaraj et al., 2022). From another side, Azolla carbohydrate and oil contents are very low and can be easily digested by the livestock, because of its high protein with low lignin content. Azolla found to

Citation: El-Deeb MM, Alazab AM, Shazly SA, Fahim HN, and Ragab MA (2024). Effects of dietary substitution of Soybean meal by dried Azolla on blood and serum parameters, productive and reproductive traits, and economic efficiency of rabbit does as well as semen quality of bucks. *Online J. Anim. Feed Res.*, 14(1): 13-21. DOI: https://dx.doi.org/10.51227/ojafr.2024.2

improves the meat quality as well as health status and longevity of livestock. Feeding Azolla to poultry birds improved the weight of broiler chicken and increased egg production of layers. Several authors used Azolla in feeding different animals (Sheep, goats, pigs, and rabbits) as a feed substitute (Pillai et al., 2001). Moreover, El-Deeb et al. (2021a) concluded that feeding Azolla for growing rabbits positively affected its growth performance without adverse influence on blood biochemical and carcass traits when substituting 40% of soybean protein with dried Azolla protein in their diets. It is known that rabbits are considered pseudo-ruminants have the capability to utilize forage protein more efficiently than other kinds of livestock. Animals' capability of utilizing nutrients via feed conversion to meat, eggs, milk, etc. depends on several factors, e.g. immunity, its health status, and environmental conditions (stress factors) inside the animals' house, feed quality, feed intake, digestion and absorption rates of nutrients as well as its elements sufficiency (Halas and Babinszky, 2014). Scientists in the field of animal nutrition are asked to achieve this by reducing production costs and obtaining the optimum productive performance with minimizing or vanishing adverse effects (Zeng et al., 2015).

The purpose of this study was to investigate the effects of substituting various levels of soybean protein with dried Azolla protein on the productivity, reproductive performance, and economic efficiency of Black Balady rabbits.

MATERIALS AND METHODS

Ethical regulation of study

All the procedures in caring for animals and methods in this study followed the Animal Welfare regulations of the Animal and Poultry Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The current study was declared by the Local Experimental Animals Care Committee's Ethics Committee and done according to the rules of Animal and Poultry Production Research Institute, Egypt which complies with ARRIVE guidelines.

Animals' management

A number of forty female and eight males of Black Balady rabbits (Egyptian local breed) aged seven and eight months (live body weight; LBW, 2860±100 g and 3330±70 g, respectively, on average) were allotted for this work. The experimental animals were divided into 4 dietary groups (10 does and 2 males each) at the rabbit research branch, El-Serw Animal Production Research Station, Animal, and Poultry Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. The four dietary treatment groups were assigned as follows: The first group fed the control diet (without Azolla), while the second, third, and fourth groups were fed diets that included 20, 30, and 40% of soybean protein substituted with Azolla protein. The rabbits housed to have three parturitions in a wired cage of the dimensions 50 x 50 x 35 Cm where fed their assigned experimental diets. Drinking water via stainless-steel nipple was available all the time in one environment condition. Hot air (80^{o}C) dried Azolla plant samples as well as other feed ingredients were analyzed for its chemical composition using AOAC (2005) methods and the formulated diets as well as calculated chemical analysis of these tested diets are presented in Table 1.

The tested diets were formulated from pellets of 4 x 12 mm in size which prepared from feed constituents bought from the local market after grounded and mixing and fed *ad libitum* according to the NRC (1994) requirements. The light was allowed for 12-14 hours/day in the animals' houses and daily morning urine and faces dropped on the floor from the cages and cleaned.

For mating purposes, each 5 does were allotted one buck who received the same specified tested diet and each doe of the treatment was transferred to the cage of its assigned buck and returned to her cage after being mated, and after 10 days from mating does ware palpated to determine pregnancy. Litter size (LS) was detected and other rearing process protocol implemented including date of birth, number of kits, stillbirth removed, and weight of kits were recorded within the first 12 hours after kindling. Litter sizes were examined also each morning for recording during the suckling period. Young rabbits were weighed for litter weight (LW) at birth, three weeks of age (21 days) and at weaning (five weeks of age; 35 days).

Reproductive and productive parameters of does

The reproductive performance traits including number of services per conception (NSC), parturition interval (PI, days), LS (No.), LW (g), and litter weaning weight (g) were examined for each doe's rabbit. The productive performance of each doe was studied in terms of litter size and weight, and mean bunny weight (MBW) was measured at birth, 21 and 35 days of age. Thereafter, daily weight gain (DWG) was calculated for the whole period from birth till weaning (35 days) during both gestation and suckling periods. Live body weight (LBW) and total feed intake (FI; Kg/doe) were recorded.

Reproductive parameters of bucks

Semen samples of the experimental bucks were collected (3 times) using an artificial vagina at the beginning, middle, and end of the experimental period. The collected samples were tested for the traits of ejaculate volume (ml), motility (%), abnormal sperm (%), live sperm (%), dead sperm (%), total mass (5/5), and sperms' concentration (x 106/ml) according to Smith and Mayer (1955).

Table 1 - Experimental ingredients chemical composition, formulated diets and its chemical analysis

Ingredients	Chemical composition (%)				Digestible energy (Kcal/Kg)
	CP	CF	EE	Ash	
Barley	11	6.5	1.9	2.5	3300
Wheat bran	15	11	4.2	7	2410
Soybean meal (44% protein)	44	7.7	1.5	6.5	3270
Dried Azolla	28	18	2	25.8	2438
Alfalfa hay	18	27	2.7	9.7	2450
Mint straw	6	35	0.78	12	1850
Formulated diets	**Azolla protein substitution percentage**				
	Control	**20%**	**30%**	**40%**	
Barley	30	27.5	25	23.5	
Wheat bran	12	12	12	13	
Soybean meal (44 %)	18	12.34	9.51	6.68	
Dried Azolla	0	5.66	8.49	11.32	
Alfalfa hay	24.0	30	35.5	40	
Mint straw	12.5	9	6	2	
Di-calcium phosphate	1.5	1.5	1.5	1.5	
Lime stone	1	1	1	1	
Salt	0.4	0.4	0.4	0.4	
Premix*	0.3	0.3	0.3	0.3	
Yeast	0.1	0.1	0.1	0.1	
Antitoxin	0.1	0.1	0.1	0.1	
Methionine	0.1	0.1	0.1	0.1	
Total	100	100	100	100	
Chemical analysis of diets on dray matter basis (%)					
Crude protein (%)	18.44	18.07	18.09	18.11	
DE (digestible energy, Kcal/Kg)	2705	2662	2637	2630	
Crude fiber (%)	13.87	14.07	14.20	13.93	
EE (Ether extract, %)	2.22	2.49	2.68	2.90	
Ca (%)	1.12	1.24	1.34	1.38	
Av. Phosphorus (%)	0.47	0.49	0.49	0.50	
Lysine (%)	0.95	0.83	0.78	0.79	
Methionine (%)	0.32	0.30	0.28	0.29	
Methionine+Cystine. (%)	0.62	0.57	0.54	0.52	

* Permix= Vitamin & Mineral mixture contained: Vitamin A, 160,000 IU; Vitamin E, 125 mg; Vitamin K3, 17 mg; Vitamin B1, 13 mg; Vitamin B2, 43 mg; Vitamin B6, 18 mg; Pantothenic acid, 85 mg; Vitamin B12, 0.17 mg; Niacin, 230 mg; Folic acid, 12 mg; Biotin, 0.6 mg; Choline chloride, 4300 mg; Fe, 0.37 mg; Mn, 670 mg; Cu, 56 mg; Co, 3 mg; Se, 2.2 mg and Zn, 480 mg.

Blood hematology and serum biochemical parameters

At the end of the study, three blood samples were collected from the marginal ear vein of each treatment' does in two separate tubes, one of each provided with EDTA as an anticoagulant. The tubes without EDTA were kept at room temperature, and then centrifuged at 3500 rpm for 20 minutes to separate clear serum. Afterward, blood samples examined for some hematological traits included white blood cell (WBC) concentration ($WBCx10^3$), red blood cell count ($RBCx10^6$) according to Hawkeye and Dennett (1989), Hemoglobin (g/dl) according to Tietz (1982) and HCT (%). Serum samples were used to determine serum total protein (g/dl), albumin (g/dl), globulin (g/dl) by difference (total protein – albumin), total cholesterol (mg/dl), triglycerides (mg/dl), creatinine (mg/dl), high-density lipoprotein (HDL; mg/dl) and low-density lipoprotein (LDL, mg/dl) by using commercial kits (Diamond Diagnostics, Halliston, MA, USA).

Economic efficiency

For economic efficiency estimation, the model of input–output analysis outlined by Zeweil (1996) was used for calculating the return depending on the local market prices during the experimental time. The following equations were applied: Total feed cost (EGP) = Total feed intake (Kg) x price/kg feed (EGP); Total return (EGP) = Total weight rabbits (kg) x price/kg live body weight (EGP); Net return (EGP) = Total return (EGP) - Total feed cost (EGP); Economic Efficiency (EE) = Net return (EGP) / Total feed cost (EGP).

Statistical analysis

Data were analyzed by using repeated measures of statistical software computer program CoStat (2017) upon the following model: $Y_{ij} = \mu + T_i + P_j + (TP)_{ij} + e_{ijk}$, where: μ = General mean, T_i = Treatment effect, P_j = Period effect, $(TP)_{ij}$ = Interaction of treatment and period effect, and e_{ijk} = Error. Significance among treatment means was tested at ($P<0.05$) using the Least Significant Difference Test (Snedecor and Cochran 1990).

RESULTS AND DISCUSSION

The chemical composition of Azolla used in this study was higher in CP, CF, and ash (Table 1), but lower in EE content than that reported by Anitha et al. (2016b). The high content of Azolla CF in the present study is within the range values (from 15.17 to 19.85%) reported by Kavya et al. (2014). Also, the high ash content was within the values between 26.28 and 29.17% reported by Kathirvelan et al. (2015). This variation and changes in Azolla chemical constituents among different research works might be due to changes in its dry matter content. The low EE level detected in this study falls within the range of 1.6-5.05% reported by Mohamed et al. (2018). It is worth noting that the variation in the chemical composition of different Azolla species leads to differences in feed intake which is associated with energy consumption. In this concern, Alalade et al. (2007) attributed the poorer growth rate of birds at 15% Azolla to lower feed intake and consequently the reduction in metabolizable energy.

Rabbit doe reproductive performance

Table 2 represents the studied reproductive traits of the Black Baladi rabbit' does as well as the average feed intake (FI; Kg/doe) during the three parities studied periods. There were no significant differences among tested groups regarding No. of services/conception as well as parturition intervals (days) with a superiority of 30% group over the control and other two tested groups. It is obvious that FI (Kg/doe) was the lowest in the 30% group, while the highest one was in the 20% group followed by 40% and the control groups, respectively. The substitution of 30% soybean protein with dried Azolla protein reduced FI significantly (P<0.05) by about 2.1% while replacing 20 and 40% of soybean protein with dried Azolla protein raised significantly (P<0.05) FI by about 10.5 and 4.6% compared to the control group, respectively. This reduction in FI may be due to the bigger LS (6.63) recorded for the 30% substitution group compared to 4.88 and 5.00 for the control, 20 and 40% groups. Average LW was significantly (P<0.05) superior in the 30% replacement group over all the other three tested groups from birth till weaning.

This trend was confirmed by Jiao et al. (2014) who stated that physiological status as well as other factors, e.g. energy content affects FI. In this concern, Xiccato and Trocino (2010) stated that rabbits try to adjust the intake of digestible energy (DE) throughout regulating their feed intake if fed *ad libitum*. This adjustment can be achieved when the dietary energy concentration ranges between 9.00 and 11.50 MJ DE/kg which is in line with the energy content of the present tested diets (Ranging between 11.00 to 11.32 MJ DE/Kg diet). Moreover, the does during lactation are in a negative energy balance (lower body fatness), and according to Lebas (2004), a reduction in feed utilization with very high energy diets (>2650 kcal DE/Kg) is still to be noticed. Recently, Read et al. (2015) reported that a diet with levels of different fiber types maximizes digestive health and results in a favorable better feed utilization.

Table 2 - Some productive and reproductive traits of Black Baladi rabbit does, fed different ratios of dried Azolla

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
No. of services/conception	1.25	1.25	1.38	1.38	NS	-
Parturition interval (days)	50.25	52.50	49.25	54.00	NS	-
Feed intake (Kg/doe)	23.20^{ab}	25.63^{a}	22.72^{c}	24.26^{bc}	*	1.45
Average litter size at birth	4.88^{b}	5.00^{b}	6.63^{a}	5.00^{b}	*	1.21
at 21days	4.63^{b}	4.50^{b}	6.25^{a}	4.63^{b}	**	0.75
at weaning	4.25^{ab}	4.38^{ab}	4.69^{a}	4.50^{b}	**	0.92
Average litter weight at birth	283.75^{b}	297.75^{b}	384.75^{a}	303.13^{b}	*	72.90
at 21days	1289.25^{b}	1084.38^{b}	1735.13^{a}	1174.25^{b}	**	248.51
at weaning	3083.38^{b}	2556.25^{b}	3821.75^{a}	2598.75^{b}	**	649.56

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD=Least significant difference at P<0.05; NS = not significant; *= P≤0.05; **= P≤0.01.

Rabbit does productive performance

Information in Table 3 introduces the studied productive traits of bunnies resulting from Black Baladi does in the four tested groups in terms of average live body weight and weight gain (DWG) from birth till weaning. The figures reflected that, although there were no significant differences in live body weight either at birth or at weaning among the four tested groups, the 30% replacement groups recorded the highest significant DWG during the whole experimental period. During the suckling period, it is difficult to consider the feed conversion ratio for the resultant bunnies, the improvements in weight gain may be attributed to the benefit of does from feeds and providing its growers with enough milk. In this concern, the increase in EE% (Table 1) of the Azolla groups' tested diets may be helped in increasing milk production for their bunnies. This opinion is supported by Maertens and Gidenne (2016) who reviewed that moderate dietary fat addition for reproductive females in intensive rabbit breeding systems has a favorable impact on milk production. However, such effects on LW at weaning are not very noticeable. Moreover, growth rate and feed efficiency are higher if the feed is adjusted for essential nutrients such as amino acids (Read et al. 2015) which may be available in

Azolla-tested diets than the control one. On the other hand, Bovera et al. (2012) concluded that feed additives can have little or no effects in improving rabbit performance.

In general, it is of interest to note that young bunnies started to consume diets with their dams after 3 weeks of age, thus it is important to estimate LW at this time. In this concern, Fortun-Lamothe et al. (2006) demonstrated that with high production efficiency of the female rabbit, the kits consumed 4.17 Kg of feed from 18 to 35 days (Weaning), or 34% of the exclusive doe consumption (12.2 Kg from parturition to weaning). Alalade et al. (2007) attributed the differences in weight gain to the variation in Azolla strains and nutrient composition as well as the type and physiological status of the used experimental animal.

Table 3 - Growth performance of bunnies from does Black Baladi rabbit fed different ratios of dried Azolla

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
Average bunny weight at birth	59.68	59.53	57.91	61.15	NS	-
Average bunny weight at weaning	686.5	732.09	729.19	695.94	NS	-
Daily weight gain/bunny (0-21 days)	47.89^{b}	37.46^{b}	64.30^{a}	41.46^{b}	**	10.88
Daily weight gain/bunny (21-35 days)	128.14^{ab}	105.13^{b}	149.04^{a}	101.76^{b}	*	31.94
Daily weight gain/bunny (0-35 days)	79.99^{b}	64.54^{b}	98.20^{a}	65.60^{b}	**	17.99

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD= Least significant difference at P<0.05; NS = not significant; *= P≤0.05; **= P≤0.01.

Reproductive parameters of bucks

Table 4 introduces the effect of substituting soybean protein in the diet with 20, 30, and 40% of dried Azolla protein. Generally, all Azolla tested groups recorded better significant (P<0.05) results in terms of ejaculate volume (ml), motility, abnormal sperm, live sperm, and dead sperm percentages as well as sperm concentration, while total mass was not significantly differed among the four tested groups. A similar trend was recorded by El-Deeb et al. (2021b) who attributed this enhancement in the reproductive traits of rabbit bucks to the active components of feed additives, e.g. antioxidant factors and/or sufficient supplied nutrient elements which are available in Azolla. Moreover, several studies on rabbits, in this area (Gabbar et al. 2019, Kandeil et al. 2019 and Abdel-Wareth and Metwally 2020) concluded that thyme aqueous extracts can play a major role in enhancing the semen quality of bucks due to its diverse pharmacological properties.

Table 4 - Semen quality traits of Black Baladi rabbits' bucks fed different levels of dried Azolla

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
Ejaculate volume (ml)	0.77^{c}	0.83^{b}	0.83^{b}	0.90^{a}	**	0.05
Motility (%)	63.53^{b}	75.97^{a}	81.17^{a}	77.67^{a}	**	6.90
Abnormal sperm (%)	15.04^{a}	11.67^{b}	11.07^{b}	9.15^{c}	**	1.81
Live sperm (%)	76.20^{c}	83.63^{b}	78.60^{c}	87.33^{a}	**	3.02
Dead sperm (%)	23.80^{a}	16.70^{b}	22.73^{a}	12.67^{c}	**	2.95
Total mass	3.33	3.67	4.33	4.33	NS	-
Concentration	173.53^{b}	185.17^{ab}	196.60^{a}	195.83^{a}	*	14.27

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD= Least significant difference at P<0.05; NS = not significant; *= P≤0.05; **= P≤0.01.

Blood and serum parameters

Exploring figures of blood and serum studied parameters in Table 5 showed no significant differences among tested diets regarding both white and red blood cell count (WBC & RBC) as well as hematocrit % in which the 40% Azolla replacement group recorded the highest values of all parameters. While hemoglobin % significantly (P<0.05) varied among the four tested groups and followed a similar trend to the other three hematology-studied traits.The obtained values of white blood cell (WBC) and red blood cell (RBC) counts are close to the published ranges of the same used experimental breed by Beshara et al. (2018) and El-Deeb et al. (2021b). In this domain, Moore et al. (2015) demonstrated that New Zealand white rabbit adult females' blood contents of WBC, RBC, and hemoglobin ranged from 5.2-10.6, 5.11-6.51 (x103 and 106/µL) and 9.8-15.8%. Moreover, Hct. (%) was lower than that obtained by El-Deeb et al. (2021b), except for the 40% replacement group which was within their range (24.6-29.03), but almost similar to the results of Beshara et al. (2018). Moreover, Özkan et al. (2012) stated that values of both red blood cell count and hematocrit (HCT) level in rabbits are influenced by stress, age, season, and genus. Melillo (2007) and Jenkins (2008) reported that HCT values

under 30% accompanied by a reduction in hemoglobin are evaluated as anemia. Furthermore, they also denoted that WBC counts increased in rabbits rarely indicate an infection.

Generally, serum studied traits (Total protein, albumin, globulin, total cholesterol, and creatinine) contents did not show any significant differences among the four tested groups. But, triglyceride concentration significantly (P<0.05) increased in the 20 and 40% replacement groups, while significantly (P<0.050) decreased compared to the control. The HDL content significantly (P<0.05) increased in the three replacement groups compared to the control, while LDL was not significantly differed among the four tested groups. Serum chemistry traits are in agreement and close to ranges of 5-7.5 g/dl, 2.5-4 g/dl and 2.5-4 g/dl mentioned by Anitha et al. (2016a). They also added that the mean values of serum chemicals are generally affected by the quality and quantities of protein intake and these values can be used as an indicator for non-stress factors as well as for the nutritional adequacy of dietary protein.

Table 5 - Some blood and serum parameters of Black Baladi rabbit does fed different ratios of dried Azolla.

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
Hematology traits						
White Blood Cells (WBC×10^3/µL)	6.37	4.80	6.00	8.00	NS	-
Red Blood Cells (RBC×10^6/µL)	4.95	5.75	4.61	6.56	NS	-
Hemoglobin (Hb; g/dL)	11.73^{a}	11.98^{a}	10.88^{b}	12.50^{a}	*	0.83
Hematocrit (Hct. %)	27.00	28.43	24.06	32.10	NS	-
Serum traits						
Total protein (g/dL)	6.57	6.50	7.77	7.20	NS	-
Albumin (g/dL)	3.53	3.93	3.60	3.73	NS	-
Globulin (g/dL)	3.04	2.57	4.17	3.47	NS	-
Total cholesterol (mg/dL)	156.67	128.67	135.67	137.00	NS	-
Triglycerides (mg/dL)	119.33^{ab}	135.67^{a}	111.00^{b}	124.33^{ab}	*	24.66
Creatinine (mg/dL)	0.95	0.97	0.88	0.87	NS	-
HDL (mg/dL)	16.00^{b}	22.00^{ab}	24.67^{ab}	32.33^{a}	*	11.78
LDL (mg/dL)	15.33	12.67	13.00	14.33	NS	-

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD= Least significant difference at P<0.05; NS = not significant; *= P≤0.05.

Economic efficiency

Calculations of the economic efficiency for substituting the proportion of soybean protein with a cheaper source of good nutrient composition source like Azolla protein at the rate of 20, 30, and 40% are presented in Table 6. The data generally indicated that the 30% replacement of the soybean protein group showed the best economic return and utilization of feed compared to the other two replacement groups and the control. The good return from this group may be attributed to the reduction in feed intake accompanied by the highest average weight rabbits (Kg/doe). This low-cost production technology is believed to be taken up more widely by dairy farmers particularly with fodder production shortage (Pillai et al. 2001). They reported that Azolla increased the feed conversion ratio and thus milk yield in cattle.

Katole et al. (2017) reported that the use of Azolla as a feed substitute saved about 20-25% of concentrate mixture feeding cost. Moreover, Jentzer (2008) stated that the feed conversion ratio was responsible for 30%, on average, of the feed cost margin variation, while this was only about 9 and 7% for the feed price and the sale price of rabbits, respectively.

Table 6 - Economic efficiency of does Black Baladi rabbit fed different diets of dried Azolla

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
Average feed intake (Kg/doe)	23.20^{ab}	25.63^{a}	22.72^{c}	24.26^{ab}	*	1.45
Average weight rabbits (Kg/doe)	6.167^{ab}	5.113^{c}	7.644^{a}	5.198^{bc}	*	1.53
Price / Kg feed (EGP)	5.24	5.25	5.27	5.28	NS	NS
Total feed cost / doe (EGP)	121.57^{bc}	134.56^{a}	119.73^{c}	128.09^{ab}	*	8.76
Price / Kg live body weight (EGP)	40	40	40	40	-	-
Total return / doe (EGP)	246.68^{b}	204.52^{c}	305.76^{a}	207.92^{c}	**	61.17
Net return / doe (EGP)$^{(1)}$	125.11^{b}	69.96^{c}	186.03^{a}	79.83^{c}	*	68.79
Economic Efficiency (EE)$^{(2)}$	1.03^{ab}	0.52^{c}	1.55^{a}	0.62^{bc}	*	0.61
Relative Economic Efficiency (REE)$^{(3)}$	100^{ab}	50.52^{c}	150.96^{a}	60.55^{bc}	*	59.25

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD= Least significant difference at P<0.05. EGP = Egyptian pound. $^{(1)}$ Net return (EGP) = Total return (EGP) - total feed cost (EGP); $^{(2)}$ Economic Efficiency (E.E) = Net return (EGP) / total feed cost (EGP); $^{(3)}$ Relative Economic Efficiency (REE) = (E.E / E.E of control) x 100.

CONCLUSION

It can be concluded from the obtained results that Azolla can safely and economically replace soybean protein at the rate of 30% for adult female rabbits without any adverse effects on their health status or their suckling bunnies. The study recommending the replacement of 30% of soybean protein with dried Azolla protein in rabbit diets to reduce the production costs of feeds and increase profitability.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dr. Mohamed M. El-Deeb; E-mail: deep121eg@yahoo.com; ORCID: https://orcid.org/0000-0002-5893-6763

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors' contribution

A.M. Alazab; S.A. Shazly; H.N. Fahim and M.A. Ragab performed research, taking and preparing samples. M.M. El-Deeb performed literature overview, analyzing results, editing and article writing. All authors reviewed and approved the manuscript.

Acknowledgement

The authors introduce their deep thanks to Chief Researchers Dr. A.El.M.I. El Desoky and Dr. M.M. Behara for their kind and valuable guidance, assistance, and efforts made during this study.

Consent to publish

All authors selected the "Online Journal of Animal and Feed Research" for publishing this research article because it is in the scope (animal nutrition) of the journal.

Competing interest

The authors have not declared any conflict of interests.

REFERENCES

Abo Omar JM, Daya R, and Ghaleb A (2012). Effects of different forms of olive cake on the performance and carcass quality of Awassi lambs. Animal Feed Science and Technology, 171: 167–172. https://doi.org/10.1016/j.anifeedsci.2011.11.002.

Abdel-Wareth AA, and Metwally AE (2020). Productive and physiological response of male rabbits to dietary supplementation with thyme essential oil. Animals, 10: 1844. http://doi:10.3390/ani10101844.

Alalade OA, Iyayi EA, and Alalade TO (2007). The nutritive value of Azolla (*Azolla pinnata*) meal in diets for growing pullets and subsequent effect on laying performance. The Journal of Poultry Science, 44: 273-277. http://DOI.org/10.2141/jpsa.44.273

Anitha KC, Rajeshwari YB, and Shilpashree J (2016a). Effect of supplementary feeding of *Azolla* on biochemical parameters of broiler rabbits. Indian Journal of Applied Research, 6: 605-607. https://www.worldwidejournals.com/indian-journal-of-applied-research-%28IJAR%29/recent_issues_pdf/2016/October/October_2016_1492168491__173.pdf

Anitha KC, Rajeshwari YB, Prasanna SB, and Shilpa Shree J (2016b). Nutritive evaluation of azolla as livestock feed. Journal of Experimental Biology and Agricultural Sciences, 4(6): 670-674. http://doi.org/10.18006/2016.4(Issue6).670.674

AOAC (2005). Official Methods of Analysis, 18th Edition. Association of official analytical chemists, Washington, D C. http://sutlib2.sut.ac.th/sut_contents/H125800.pdf

Beshara MM, Alazab AM, Fahim HN, El Desoky AEIMI, Ragab MA, El-Fhhat AM, and El-gamal AA (2018). Effect of early dietary supplementation of probiotic and feed restriction post weaning on productive and economical performance of growing rabbits. Egyptian Journal of Rabbit Science, 28(1): 195 -222. http://DOI.org/10.21608/ejrs.2018.46510

Bovera F, Lestingi A, Marono S, Iannaccone F, Nizza S, Mallardo K, de Martino L, and Tateo A (2012). Effect of dietary mannan-oligosaccharides on *in vivo* performance, nutrient digestibility and caecal content characteristics of growing rabbits. Journal of Animal Physiology and Animal Nutrition, 96: 130–136. http://DOI.org/10.1111/j.1439-0396.2011.01134.x

CoStat (2017). CoStat Ver. 6.451, Copyright(c) 1998-2017, CoHort Software. https://www.cohortsoftware.com/DownloadCoStatPart2.html

DeFrank J (1995). Response of Wetland Taro Yield and Weeds to Preplant Establishment of Azolla in Hawaii. Colloquia Workshops 92nd Annual Meeting of the American Society for Horticultural Science. 40th Annual Congress of the Canadian Society for Horticultural Science. Montréal, Québec, Canada, 30 July–3 August 1995. Horticultural Science, 30(4): 912. https://doi.org/10.21273/HORTSCI.30.4.912F

El-Deeb MM, Fahim HN, Soheir A Shazly, Mona A Ragab, Alazab AM, and Beshara MM (2021a). Effect of partially substitution of soybean protein with azolla (*Azolla pinnata*) on productive performance and carcass traits of growing rabbits. Journal of Animal and Poultry Production, Mansoura University, 12 (6): 197-203. http://DOI:10.21608/jappmu.2021.182678

El-Deeb MM, Fahim HN, Alazab AM, Nahla A EL.Razik, Beshara MM, and Almwafy AA (2021b). Effect of adding citric acid (CA) to corn distillers dried grains with solubles (DDGs) diets on reproductive and productive performance of rabbits. Egypt Poultry Science, 41(II): 369-392. http://DOI.org/10.21608/epsj.2021.182515

Fasuyi AO, and Aletor VA (2005). Protein replacement value of cassava (*Manihot esculenta*, Crantz) leaf protein concentrate (CLPC) in broiler starter: Effect of performance, muscle growth, haematology and serum metabolites. International Journal of Poultry Science, 4: 339-349. http://DOI.org/10.3923/ijps.2005.339.349

Fortun-Lamothe L, Lacanal L, Boisot P, Jehl N, Arveux A, Hurtaud J, and Perrin G (2006). Utilisation autour du sevrage d'un aliment riche en énergie et en fibres: effet bénéfique sur la santé des lapereaux sans altération des performances de reproduction des femelles. [Study of the effect of bunuim incrassatum on rabbit growth and some haematological, hormonal and mammary gland histology structure in lactating rabbits]. Cuniculture Magasine, 33: 35-41. http://hdl.handle.net/123456789/14316

Gabbar MA, Ahmed RR, Kandeil MA, Mohamed AEH, and Ali SM (2019). Administration of ginger and/or thyme has ameliorative effects on liver and kidney functions of V-line rabbits: Histological and biochemical studies. Journal of Animal Physiology and Animal Nutrition, 103: 1758-1767. https://doi.org/10.1111/jpn.13166.

Halas V, and Babinszky L (2014). Role of dietary polysaccharides in monogastric farm animal nutrition. In: Polysaccharides: Natural Fibers in Food and Nutrition, edited by Noureddine Benkeblia, CRC Press, Taylor and Francis Group, 2014. Chapter 18, Pp. 436-437. https://www.taylorfrancis.com/chapters/edit/10.1201/b17121-23/

Hawkeye CM, and Dennett TB. (1989). A Color Atlas of Comparative Veterinary Hematology. Wolf Publishing Limited, London, UK. https://doi.org/10.1111/j.1939-165X.1989.tb00532.x

Issa K, and Abo Omar J (2012). Effect of garlic powder on performance and lipid profile of broilers. Open Journal of Animal Science, 2: 62-68. http://dx.doi.org/10.4236/ojas.2012.22010

Jenkins JR (2008). Rabbit diagnostic testing. Journal of Exotic Pet Medicine, 17: 4-15. http://doi:10.1053/j.jepm.2007.12.003

Jentzer A (2008). Performances moyennes des élevages cunicoles en 2007. Présentation rapide des résultats RENACEB et RENALAP. [Average performance of rabbit farms in 2007 Quick presentation of RENACEB and RENALAP results]. CunicuMag, 35: 39-44. http://www.cuniculture.info/Docs/Magazine/Magazine2008/mag35-039.htm

Jiao S, Maltecca C, Gray KA, and Cassady JP (2014). Feed intake, average daily gain, feed efficiency, and real-time ultrasound traits in Duroc pigs: I. Genetic parameters estimation and accuracy of genomic prediction. Journal of Animal Science, 92: 2377-2386. http://DOI.org/10.2527/jas.2013-7338

Kandeil MA, Mohamed AEH, Gabbar MA, Ahmed RR, and Ali SM (2019). Ameliorative effects of oral ginger and/or thyme aqueous extracts on productive and reproductive performance of V-line male rabbits. Journal of Animal Physiology and Animal Nutrition, 103: 1437-1446. http://doi:10.1111/jpn.13147.

Kathirvelan C, Banupriya S, and Purushothaman MR (2015). Azolla-an alternate and sustainable feed for livestock. International Journal of Environmental Science and Technology, 4(4): 1153-1157. https://api.semanticscholar.org/CorpusID:90867315

Katole ShB, Shweta R Lende, and Patil SS (2017). A Review on Potential Livestock Feed: *Azolla*. Livestock research international, 5(1): 1-9. https://web.archive.org/web/20180411141009id_/http://jakraya.com/journal/pdf/15-lriArticle_1.pdf

Kavya K, Rabhu TMP, Loridoss RGG, Chandrapal Singh K, and Ajeshwari YBR (2014). Nutritional evaluation of Azolla (*Azolla pinnata*) and its supplementary effect on *In vitro* digestibility of total mixed ration. Mysore Journal Agricultural Sciences, 48 (3): 413-419. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20153302187

Lebas F (2004). Reflexions on rabbit nutrition with a special emphasis on feed ingredients utilization. In: Proc. 8th World Rabbit Congress, Puebla, Mexico, pp. 686-736. https://api.semanticscholar.org/CorpusID:86182662

Maertens L, and Gidenne T (2016). Feed efficiency in rabbit production: Nutritional, technico-economical and environmental aspects. 11th World Rabbit Congress - June 15 - 18, 2016 – Qingdao - China. Feed and Feeding, 337-351. https://hal.archives-ouvertes.fr/hal-02046863

Melillo A (2007). Rabbit clinical pathology. Journal of Exotic Pet Medicine. 16: 135-145. http://doi:10.1053/j.jepm.2007.06.002

Mithraja MJ, Johnson M, Mahesh M, Paul ZM, and Jeeva S (2011). Phytochemical studies on *Azolla pinnata* R. Br., *Marsilea minuta* L. and Salvinia molesta Mitch. Asian Pacific Journal of Tropical Biomedicine, 1: S26–S29. https://doi.org/10.1016/S2221-1691(11)60116-0

Mohamed AM, Elnemir SE Abd El-Mounem SM, and Abo El-Maati SM (2018). Azolla fern as untraditional resource of protein. Zagazig Journal of Agricultural Research, 45(4): 1345-1355. http://doi.org/10.21608/zjar.2018.48582

Moore DM, Zimmerman K, and Smith SA (2015). Hematological assessment in pet rabbits: Blood sample collection and blood cell identification. Veterinary Clinics of North America: Exotic Animal Practice, 18(1): 9-19. http://doi:10.1016/j.cvex.2014.09.003.

Nasir NA, Kamaruddin SA, Zakarya IA, and Islam AK (2022). Sustainable alternative animal feeds: Recent advances and future perspective of using azolla as animal feed in livestock, poultry and fish nutrition. Sustainable Chemistry and Pharmacy, 25:100581. https://doi.org/10.1016/j.scp.2021.100581

NRC. 1994. Nutrient Requirements of Poultry. 9th rev ed. Nat. Acad. Press Washington. https://vetbooks.ir/nutrient-requirements-of-poultry-9th-revised-edition/

Özkan C, Kaya A, and Akgül Y (2012). Normal values of haematological and some biochemical parameters in serum and urine of New Zealand White rabbits. World Rabbit Science, 20: 253-259. http://doi:10.4995/wrs.2012.1229

Pillai K, Premalatha S, and Rajamony S (2001). Azolla – A sustainable feed substitute for livestock. Small Enterprise Development and Management Extension (SEDME), 28(4): 51-58. https://doi.org/10.1177/0970846420010405

Read T, Combes S, Gidenne T, Destombes N, Grenet L, and Fortun-Lamothe L (2015). Stimulate feed intake before weaning and control intake after weaning to optimise health and growth performance. World Rabbit Science, 23(3): 145-153. https://doi.org/10.4995/wrs.2015.3977

Roy D, Kumar V, Kumar M, Sirohi R, Singh Y, and Singh JK (2016). Effect of feeding *Azolla pinnata* on growth performance, feed intake, nutrient digestibility and blood biochemical's of Hariana heifers fed on roughage-based diet. Indian Journal of Dairy Science, 69(2): 190-196. https://epubs.icar.org.in/index.php/IJDS/article/view/50583/pdf_199

Samad FA, Idris LH, Abu Hassim H, Goh YM, and Loh TC (2020). Effects of Azolla spp. as feed ingredient on the growth performance and nutrient digestibility of broiler chicken. Journal of Animal Physiology and Animal Nutrition, 104(6):1704-1711. https://doi.org/10.1111/jpn.13345

Smith JT, Mayer DT (1955). Evaluation of sperm concentration by the haemocytometer method. Fertility and Sterility, 6: 271-275. http://doi.org/10.1016/s0015-0282

Snedecor GW, and Cochran WG (1990). Statistical Methods 8th Ed. Iowa State Univ. Press Ames, Iowa, U.S.A. 609. The Journal of Agricultural Science. 115(1): 153-153. http://doi:10.1017/S0021859600074104

Thangaraj M, Saravana BP, Thanasekaran J, Joen-Rong S, Manubolu M, and Pathakoti K (2022). Phytochemicals of algae, *Arthospira platensis* (spirulina) *Chlorella vulgaris* (chlorella) and *Azolla pinnata* (azolla). GSC Biological and Pharmaceutical Sciences, 19(2): 23-43. https://doi.org/10.30574/gscbps.2022.19.2.0167

Tietz NW (1982). Fundamental of clinical chemistry. Edition by Nobert Sounder Comp., Philadelphia, PA, USA. https://search.worldcat.org/title/12096260

Tubb C, and Seba T (2021). Rethinking food and agriculture 2020-2030: the second domestication of plants and animals, the disruption of the cow, and the collapse of industrial livestock farming. Industrial Biotechnology, 17(2): 57-72. https://doi.org/10.1089/ind.2021.29240.ctu

Xiccato G, and Trocino A (2010). Energy and protein metabolism and requirements. In: Nutrition of the rabbit. C De Blas & J Wiseman (eds), CABI publication, Wallingford; UK, pp. 83-118. https://doi.org/10.1079/9781845936693.0083

Zeng QF, Cherry P, Doster A, Murdoch R, Adeola O, and Applegate TJ (2015). Effect of dietary energy and protein content on growth and carcass traits of Pekin ducks. Poultry Science, 94(3): 384-394. http://doi.org/10.3382/ps/peu069.

Zeweil HS (1996). Enzyme supplements to diets growing Japanese quails. Egyptian Poultry Science Journal, 16: 535–557. Google Scholar