UTILIZATION OF (DUCKWEED) IN IRRIGATION WATER AS A REPLACEMENT OF SOYBEAN MEAL IN THE LAYING HENS DIET: PERFORMANCE AND EGG QUALITY

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Abstract

Water lentils (Duckweed)(DW)(*Lemna gibba*), in irrigation ponds, was evaluated by replacing two levels of soybean meal (SBM) on quality and performance of egg laying hens at 54 weeks of age. A total of 72 Lohmann white laying hens were randomly allocated into 3 treatments with 6 replicates / treatment 4 hens/ replicate in a randomized complete block design, with 6 blocks each 3 pens. Treatments were: control group (DW0%) with (SBM) as the only source of protein, T1 (DW10%) and T2 (DW20%) were duckweed replaced 10 and 20% of SBM for 9 weeks. There were no significant differene observed among the treatments of diet of change in body weight, conversion ratio of feed, mortality rate and weight of the egg. Replacement with (DW20%) significant decrease (P <0.05) in feed intake, egg laying rate and mass of egg. The dry albumin% in (DW10%) was decreased significantly from 7 to 9 wks and in the total period. Yolk pigmentation was highly *(*P <0.001) improved by the replacement. Blood spots were increased (P <0.05) with (DW20%). Duckweed grown in good quality irrigation water can be fed up to 10% replacement of SBM as a source of protein without negative effect on hen performance and egg quality in addition to profitability.

Key words: Duckweed, irrigation water; soybean meal; egg production; yolk pigmentation.

INTRODUCTION

Duckweed (Lemna gibba) which is also called water lentil is one of the four plants species that is from the botanical family named as Lemnaceae. It is also classified as higher plant species or it can also be called as macrophytes and mistaken for algae. They are also the simplest and smallest plants of the world (Hilman & Culley, 1978) distributed worldwide in ponds, ditches lakes, and canals forming green mats. High salt concentrations (up to 4000 mg/liter total dissolved solids) and wide range of pH (4.5-8.5) can be tolerated by these plants (Zimmo, al., 1995). Shammout and Zakaria, (2014) reported that these plants have an important role in purifying the irrigation water. It was used as a natural bioremediation agent for the treatment of water (Shammout et al., 2008). The prevailing conditions of climate and nutrient content of water are the main factors which can have significant impact on duckweed composition. Fresh duckweed contains 92 to 94% water; if ideal conditions are present during its growth and harvesting. If ideal environment is given to duckweed it will have 5 to 15 % of fiber and 35 to 45 % of protein is present on dry weight (Skillikorn et al., 1993). Goopy and Murray (2003) confirmed that duckweeds can absorb nutrients from the waste and drained water forming biomass rich in protein, carbohydrates, and pigments suitable for feeding domestic animals and fish. For livestock it is also a decent source of minerals and vitamins (Landolt & Khandeler 1987; Men et al., 2001). Ammonium ions as a useful N source and nitrogen as a protein are stored by duckweeds when nitrogen is present in high level in water (Mkandawire et al., 2005). Nafea and Zyada (2015) found that *Lemna gibba* can absorb nutrients from water and forms biomass rich in nutrients especially protein. Leng et al., (1995) stated that the crude protein level of duckweeds depends primarily on the level of nutrients concentrations in the water, and on the species involved and the nitrogen supply. Well balanced profile of amino acids is one of the significant quality of the duckweeds (Landolt & Kandeler 1987). It also has the high concentration of the lysine, methionine and amino acids which is very essential (Skillicorn et al., 1993). Many authors (Haustein et al., 1990b, 1992 and 1994; Islam et al., 1997; Rodriguez et al., 1999, Leng 1999; and Samnang, 1999) have recognized the use of duckweeds in manufacturing of poultry feed even after the existence of first limiting factor which is moisture content. Duckweed could be a substitute of the soybean meal up to 15 % of the total food and fish meal in the laying hen's diet if it is of high quality which contains 30 to 40 % of proteins with low ash and high carbohydrate. It will also be good for healthy egg production, improvement of proteins in egg content and high pigmentation of yolk (Haustein et al., 1990b). O'Neil et al., (1996) also found that there was an improvement in yolk pigmentation from the addition, which is due to presence of 13% of duckweed in the laying hen's diet. There is a high strength of trace minerals in duckweed like phosphorus, some pigments such as xanthophylls, carotene etc and potassium which is helpful for the production of an dietary supplement for chicken (Haustein et al., 1990b, 1992 and 1994).

In the total production cost required for raising poultry there is 60 % involvement of cost of animal feed which is also connected with the high cost of sources of proteins like soybean meal (Ministry of Agriculture, 2014). Demand for an alternative source of protein required for feeding which is also local has been increased (Shammout & Zakaria, 2015b). Using local protein sources which have a same level of quality as compared to the meal of soybean which can be replaced by imported sources can be the solution to reduce production costs. *Lemna gibba* is an important specie of *Lemna* that has been discovered in the waters of Jordan (Al-Eisawi, 1982). Other studies were conducted on this species in Jordan for the purpose of evaluation of the role of duckweed in purifying ponds for irrigation of farm which can act as a bioremediation agent for a natural water and a best source of protein for poultry (Shammout & Zakaria, 2014, 2015b). There is not a single evidence found in Jordan about the replacement of duckweed as protein for the diet of egg laying hens. Replacement of this specie with SBM can be a main issue and barely received any attention.

Therefore, the present study is the first in Jordan; and it was aimed out to utilize the water lentils growth in the ponds of irrigation water by investigating it's effect of replacing percentages of costly conservative source of protein such as soybean meal with the optimum level of unconventional water lentils (duckweed) on the functioning, egg production and egg laying hen's quality based on water quality, nutritive value of duckweed and cost.

MATERIALS AND METHODS

Collection and preparation of duckweed samples and water quality analysis:

Duckweed was manually collected from irrigation water ponds in central Jordan Valley; in particular the farms of Tal al-Ramleh, Wadi al-Abyad, and Ghor Kabid in Jordan by using pool skimmer nets. As the duckweed plants is in the form of floating mat with no unity in the structure, therefore it is very easy to collect them. Fresh duckweed was transported wet to the poultry laboratory in the School of Agriculture at the University of Jordan. Debris associated with the plant were removed then, the duckweed was air dried to approximately 40% moisture for 3 successive days, and then drying was completed in a forced air oven to around 6% moisture. The dehydrated duckweed was kept at room temperature in absorbent bags which are tied to be ready for further examination. Samples of the dried duckweed were analyzed for chemical composition (AOAC, 2005) prior to its use as a feed ingredient for DM (dry material), crude fat, crude fiber, crude protein and minerals (Table 1a). On time of plant sampling, twenty samples of water were gathered for investigation to detect water quality, such as Cl ,Ca , K, Mg, PO4, SO4, Zn, NO3, Cu, Pb and Cd (Table 1b), (Shammout & Zakaria, 2015a). The research was organized throughout the spring season, which is considered the vegetative period of the duckweed plant and continued through summer.

Birds, experimental design, and diets formulation:

The trial was conducted in open wire cage system housing, at the University of Jordan/ School of Agriculture. A total of seventy-two white egg laying hens of Lohmann breed which are 54 weeks old and were distributed randomly to 3 treatments, every treatment consists of 6 replicates cage as blocks, with (4 birds/replicate cage), and fed three different diets in a randomized complete block design, 3 cages represented one block for a total of 6 blocks, with one block per row of cages. Formulated diet was mainly based on corn and soybean meal. Dietary treatments were T1 (control), were soybean meal was handled as the only protein source, (2716.6Kcal/Kg) ME, 47.6% protein, a layer diet containing 10% duckweed (T2), and 20% duckweed (T3) replacing the same percentage of soybean meal in the diet. The experimental diets were formulated in accordance with recommendations of the manual of the Lohmann management guide (2005) at the stage of production taking into consideration the requirements of NRC (1994) for laying hens as presented in (Table 2). The diet was fed in mash form and representative feed samples were ground for chemical analysis. Each cage (40×40 cm) was provided with a nipple drinker and a feeder. Water and feed were supplied *ad-libitum*, and feeders among the different cages which were separated by a wooden sheet to prevent mixing of treatments of diet. Each hen was weighed at the beginning of the trial and 4 hens with similar average weight were housed in one cage. Hens were placed in the cages for one week before the trial started to adapt them for the feed and the environment and no experimental data was collected at this stage. The experiment with data collection lasted for 9 weeks (54-63wks of age). Programmable lighting setting was provided in the bird house with 10 hours of day length and during period of experiment, 6 hours light were provided. Inside house temperature was maintained at 20°C and 55-60% relative humidity. In the experimental period duration, identical and management care were provided to the birds with proper sanitation and hygienic measures. The experimental hens were treated according to standards for the caring treatments of animals by ensuring that the guidelines are properly followed given by the Jordanian Society for the Protection of Animals (1997).

***Data collection for production and egg parameters*:**

The consumption of feed was recorded on weekly basis by subtracting remaining feed from the whole feed delivered and adjusted for mortality. Representative feed samples were collected and ground for chemical analysis (AOAC, 2005). Egg production was collected, weighed, classified and recorded daily with remarks on the cracked and blooded eggs. Calculations were based on a hen-day and hen-housed basis. Mass of egg was determined by the equation (egg production × egg weight). Layers were weighed individually every two weeks until the termination of the investigational period for assessment of changes in the body weight. Feed efficiency per dozen of eggs was determined by calculating the ratio between feed consumed (g) and total eggs produced (g) over a period of time. Hen mortality was recorded daily and feed intake and egg production were corrected for mortalities.

***Measurements of egg quality*:**

Samples of 18eggs/treatment (3/replicate) were randomly collected bi-weekly for external and internal quality parameters by the separation, weighing and determining egg components (% wet and dry albumin, yolk and shell) after drying at 50-55°C for 48hrs. Weight measurements and separation of components of egg were according to the suggestion given by Chowdhury (2000). Eggshell thickness was gauged by shell thickness micrometer (Griffen & Goerge Ltd, Japan), as the average value of three different locations present on the egg. Haugh unit score was concluded using the Haugh (1937) methods through which the height of the albumin can be measured and Haugh unit can be calculated on the basis of egg weight using tripod micrometer height gauge, following the equation: Haugh units=100log (albumin height (mm) +7.57to -1.7 and weight of egg 0.37(g). The colour of Egg yolk was determined with the help of comparison with the 15 point scale of Roche Yolk colour fan (F, Hoffmann-La Roche and Co. Ltd. Basic, Switzerland).

***Statistical analysis of data*:**

Randomized and complet desing block has been used for data analysis. There were 6 blocks each containing 3 replicate pens (4birds/pen) with 3 dietary treatments; each treatment represented once in each block. Treatment effects were evaluated as a one-way repeated measures ANOVA using the MIXED procedure in SAS (v. 9.3, 2010) with dietary treatments as the main source of variation among measured parameters. Pairwise comparisons were used to estimate the significance of differences between least square means. Changes were considered significant where ($P < 0.05$), unless otherwise specified.

***Economic Assessment*:** Economic assessment was carried out using the price of feed ingredients at the time the trial was carried out to compare the cost of the diverse actions when levels of soybean meal were replaced by the duckweed plant.

RESULTS

Duckweed (*Lemna gibaa*) *and water quality analysis*:

The nutritional values of the duckweed plants were concluded in the light of the standard methods given by AOAC (2005). Fresh duckweed samples contain almost 94% water and 6% of DM. The average values of nutritive on dry matter basis are shown in (Table 1a), (Shammout and Zakaria, 2015b). The analyzed water quality parameters were according to the allowed limits set by the Jordanian Standard (JS 893/2006) for irrigation. The different minerals such as (K, Cl, Ca, SO_4, Mg, and NO_3) and the substantial metals (Zn, Cd, Pb and Cu) were according to the limites of Ideal Detection as shown in Table1b. This applies also for water pH (8.1) EC (Electrical conductivity),(1.62mS/cm), BOD_3 (Biological Oxygen Demand)(0mg/l) and COD (Chemical Oxygen Demand)(0mg/l), (Shammout & Zakaria, 2014, and 2015a,b).

Laying performance:

The effect of replacing 10 and 20 % of soybean meal with the same percentage of duckweed is shown in (Table 3). Feed intake decreased highly significant (P =0.002) through the experiment intervals and during the total period when replacing 20% of soybean meal with the duckweed, while there was little impact on body weight change with no significant difference. Egg laying rate% seemed to be significantly (P <0.05) reduced in hens receiving the 20% duckweed, the 10% was very close to the control group. It is also evident that egg production decreased with age through the total period of the trial, which is a normal trend in laying hens. Feeding at 20% duckweed caused a decrease in the daily egg-laying rate, while it was variable with egg weight since it was noted that through the 7-9 weeks of the trial it was higher than the 10 % and the control.

The ratio of feed conversion was almost same in all groups of dietary supplements and there was not a significant difference observed. Mass of egg was significantly (P <0.05) decreased by the 20% duckweed supplementation in each period of the trial. Control group and the 10% added group showed higher mass of egg than the 20% group. Mortality rate presented a significant trend (P <0.05) in the period (7-9) wk in the 20% duckweed supplementation group, but not in other periods nor in the total period of the trial.

Egg quality parameters:

The impact of supplements if duckweed on quality of egg are given in (Table 4). There is no significant changes among the three different treatments were shown in the Haugh unit, shell thickness, wet and dry shell %, and wet and dry yolk%. Wet albumin %, showed no significant dietary effects, contrary to dry albumin %, which showed a significant effect (P <0.05) between 7 and 9 weeks and in the whole period of the trial. It decreased with the 10% supplements of the duckweed (3.79, 3.77%) compared to the control (4.12, 4.02). Yolk Roche color score was highly significant (P =0.0001) in each period of the trial and through the total period with the different treatments (it increased from 5.71, 6.4 to 6.86).Yolk color increased with increasing the percentage of duckweed supplement in the diet.

The blood spots % (Table 5) had a significant effect in the 1-3weeks period and through the total period (1-9)week, with the 20% supplements compared to the 10% and the control groups in the total period of the trial (6.13 vs. 0.75, 1.15). Grades of eggs were not affected by the dietary supplements but there was a slight and clear shift in the grades of the egg which is downward with the presence of the two levels of duckweed in the diet.

DISCUSSION

Duckweed Analysis:

Duckweed samples were analyzed and determined according to (AOAC, 2005) (Table1a), (Shammout & Zakaria, 2015b). Crude protein% was 26 on 6% DM, although other researchers reported higher crude protein content (Chowdhury et al., 2000; Anderson et al., 2011; Akter at al. 2011). It is also evident that that the difference in the duckweed content is significant and it depends on the location, season, environment and species and the content of nutrient of water (Khadaker et al., 2007, Chantiratikul et al., 2010) which is mostly nitrogen concentration (Leng, 1999), water pH (Goopy & Murray, 2003), different management applied, and if it was collected from a waste lagoons or natural water, as in this study. Results of crude protein level indicated that duckweed has good protein % to compliment with soybean meal in satisfying protein requirement of layers since protein is very important for body tissue synthesis and egg production.

The crude fiber % in our study was 5.2 % which is considered desirable and suitable for hen feeding since it can be easily digested. Other results reported by different researchers were either lower or higher %, (Chara et al., (1999) (2.8%), Leng et al., (1995) (9.0%), Russof et al., (1980) (9.45%). While Men et al., (1995) reported more elevated crude fiber % (18.7%) and Khanum et al., (2005) reported (12.3%). Variations of results are due to conditions of growing, harvesting and different duckweed species.

The observed atmosphere content was 3.1% in the extract and the found value in the present research is greater than the reports given previously (Khan et al., 2002a) who recorded 2.4% fat, while, Khandaker et al., (2007) reported a higher percentage of (5.06%). Ether extract% inclined to growth with increase in the level of duckweed in the diet as shown in Table 2. It is possible that this could decrease feed intake and degree of delectableness (De Silva & Anderson, 1995), which leads to a reduction in egg laying rate and less egg quality. So it is more important in future studies to determine the fatty acids profile of this species in order to include this duckweed in the poultry rations.

Calcium content in duckweed was 4.3%, which is considered relatively a high percentage compared to SBM (0.27). Becerra et al., (1997) reported 1.1% compared to 0.4% SBM of DM, whilst Men et al., (1995 and 1996) found calcium content of (0.7% in duckweed of DM). There is the variation in nutrients level which is because of the medium in which the duckweed plant species are grown (Mwale & Gwaze, 2013). Phosphorus % of dry matter content was 0.86% which is comparable to 0.62 % DM for SBM while, Becerra et al., (1997) and Men et al., (1995; 1996), reported 0.5% P on DM basis. 1.5 % of the Duckweed weight is accumulated as the presence of phosphorus in as a nutrient in rich water which is considered to be a normal thing (Leng, 1999). Chlorine % is higher than SBM this is related to the quality of the water where this species is grown, which is within the permitted levels. The mineral concentration of the growth medium is very important factor for the nutritional content of duckweed, while species and geographical location are less important. It is also imperative to calculate the mineral sketch of the plant earlier to the diet preparation because of the high mineral content which might lead to detrimental effect.

***Body Performance and egg Production*:**

The effect of supplementing the diet with duckweed to replace percentages of soybean meal is presented in (Table 3). No significant variances have been observed in weight of the body and conversion ratio of feed among treatments during the different phases. The results which are non-significant for changes in weight of the body are according to Hamid et al., (1993) who has experience of feeding Lemna meal to ducklings and observed minimum variations in weight gain, also Akter et al., (2011) recorded same results when *Lemna minor* meal was used as a dietry supplement for laying hen. However, feed intake was decreased significantly (P =0.002) by duckweed supplementation up to 20% replacement of soybean meal, although the crude fiber content in the diet used in this trial was not high (3.11%). The highest feed intake was for the control and the 10% duckweed through the different periods of the trial. These results are in consistent with Haustein et al., (1990) who described important variances in consumption of feed between the control, the *Lemna* 15% (P <0.02) group and the *Wolffia* 15% (P<0.03) group, where the *Lemna* group showed a slight decrease in feed intake. Chantiratikul et al., (2010) also reported reduction of feed intake (P <0.05) when CP was completely substituted by CP from *Wolffia* meal or when using 12% nutritional *Wolffia* meal in the diet, contrary to ONeil et al., (1996). ONeil et al., (1996) found that characteristics of production and intake of feed were unchanged by supplementing feed up to 13% duckweed in the laying hen's diet. Akter et al., (2011), similarly reported no significant variations in feed intake when *Lemna minor* meal was added to laying diet. The higher percentage of duckweed in diets (20%) might have suppressed the appetite due to unspecified anti-nutritional factors or compounds which likely to be inhibiting digestion and metabolism(Goopy & Murray, 2003) or due to differences present in digestible protein content. Feed conversion was better numerically but not significant with the higher concentration of the duckweed during the total period of the trial due to the decrease in feed intake and consequently lower body weight. The findings are according to (Haustein et al., 1990 and Hamid et al., 1993). Presence of some anti-nutritional factors can explain the results that is limited growth and intake when high level feeding took place (Goopy & Murray 2003).

Mortality % was significant (P <0.01) in the period between 7-9wks of the study with the replacement of 20%, but it was corrected in the whole period without any significant effect between treatments, mortality within treatments ranged from 1.03, 2.04, 7.11% respectively. This implies that 20% replacement of SBM with duckweed had an negative effect on layers since it increased mortality percentage, although birds were reared in cages under good management conditions. Same results were recorded by Hassan & Edwards (1992) who mentioned rate of mortality which is of 80%. It is the highest feeding rate when they involved *S. polyrrhiza* and *L. perpusilla* up to 30 g DM/kg in Nile tilapia's diet, where these species have 23% CP, in contrast to Faskin et al., (1999), who used (50% CP) and documented no significant growth in mortality, as compared to the control group, even with100% replacement. Previous reports by No significant differences has been shown in the mortality of the laying quals where the Wolffia Arrhiza meals has been used. It may be due to the decrease in the protein component of the plant material. There can be the exposure to the elements of the anti-nutrition in feed increase and thus negative results of growth responses can be observed (Goopy & Murray 2003). Inconsistencies among results were due to species differences of duckweed and birds used, or other environmental conditions of the different trials.

Egg laying rate% significantly (P <0.05) was reduced in birds that received the 20% duckweed while those received the 10% produced eggs at a rate close to the control birds (Table 3). During the 7-9wks(P= 0.001) and during the whole period of the trial (P < 0.01) laying rate decreased significantly in the 20% DW group.

The output of mass of egg was comparable with the 10% duckweed group but it was significantly (P <0.05, 0.01) reduced to the higher level, probably due to the low rate of production. In turn, this is a reflection of feed consumption that decreased with a higher percentage of duckweed that might have some, anti-nutritional factors which depress bioavailability and utilization of nutrients in the digestive tract and affect performance. The absence of a clear reduction in egg weight suggests that body reserved were mobilized to maintain egg production (Paterson et al., 2000) since there was a slight decrease in body weight.

Egg Quality:

External and internal egg quality characteristics are shown in Table 4 and 5. Dry albumin % (Table 4), was significant (P <0.05) with the 10% duckweed compared to control and 20%, in contrast to findings reported by Akter et al., (2011). Haugh unit score, the key indicator of interior egg quality, was not influenced by diets supplements, but it decreased numerically with the increased level of duckweed. There is highly significant improvement which is p=0.0001 in the color of yolk with duckweed's increasing level in the diet. It also indicates that the specie of Lemna Gibba contains the essential amount of pigments which is enough to give attractive darker yolks. This agrees with (O'Neill et al., 1996; Nolan et al., 1997; Akler et al., 2011; Anderson et al., 2011; Chantiratikul et al., 2010; Suppadit, 2012). Without referring to the color score of yolk Haustein et al., (1990) reported that there is increase in pigmentation which is p <0.01 when Wolffia (150 g/kg) and Lemna gibba (150g/kg) are the species which are included in the diet. There are high pigment's concentration especially of xanthpphylls which is 261-1000 mg/kg and beta carotene which is 120 – 627.2 mg/kg (Haustein et al., 1990, Skillcorn et al., 1993, Hanczakowski et al., 1995). High contribution of leaves and anatomical structure of the plant is responsible for the high concentrations to its total biomass (Hanczakowski et al., 1995: Chantiratikul et al., 2010b). The economic value of the duckweeds as the ingredients of diet is with the help of pigmentation which is an important factor since it is desirable commercially.. Blood spots % was significant (P <0.05), in the 1-3weeks period and through the total period (1-9) weeks, and it recorded high value with the 20% supplements compared to the other two groups (6.13 vs.0.75, 1.15). Blood spots are usually formed due to tissue irregularity in the hen causing a small amount of blood to be deposited in the egg. It usually occurs due to vitamin A or K deficiency, but since this was not present in the diet and not seen in other groups, then it might be due to stress as a result of high amount of the duckweed inclusion levels in the diet

(USDA, 2000). Shell thickness% and cracked egg % were not significant between treatments since there was no difference in Ca% between diets which affect the shell strength and this reflects the insignificant results of egg weight within the different treatments.

***Economic assessments*:**

The price of feed was highest for the control group (243.42 JD/ton feed), (Table 2) and it tended to decrease with increasing the level of *duckweed* substitution. Production cost calculation was based only on feed cost. Duckweed is not a conventional feed and its price was only estimated for collection and drying. Since results indicated that 20% duckweed in the diet did not improve performance and it decreased egg production, therefore the comparison is between the control and the 10% which gave 5JD/ton profit. If it is assumed that on the average a feed processing unit produces a 200metric ton of feed/day, 200×30days= 6000metric ton/month, this gives 6000×5 JD= 30.000JD. This indicates the profit that will be gained in using the duckweed to replace certain percentages of expensive soybean meal.

***Conclusions*:**

Duckweed by knowing the quality of water where it grows in and its chemical analysis, can be used as a source of protein and pigment to replace 10% of SBM in laying hens ration, with no harmful effect on production performance and egg quality, while increasing the level up to 20% decreased the reproductive performance.

The advantage from feeding duckweed to laying hens would lie in its use as a source of pigment to make eggs more attractive for consumers, and also it is a very good source of minerals besides decreasing feed cost.

Since this trial is the first in Jordan to use this species, further work is needed to evaluate other percentages of duckweed in diets of laying hens. Environment related to growth and quality investigation is very important to establish the economic value of this feed for use in future formulations.

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Appendices

Table 1a. % Nutrient composition of duckweed (*Lemna gibba*) and soybean meal

Nutritive value	Contents on dry matter (%) for duckweed[1]	Contents on dry matter (%) for soybean meal
Dry matter	6.00	88.00
Water content	94.00	12.00
Protein (%N× 6.25)	26.00	47.60
Crude fiber	5.20	4.05
Ether Extract	3.10	2.20
Energy (Kcal/Kg)	2913	2337
P	0.86	0.62
K	2.40	2.06
Ca	4.30	0.27
Mg	0.88	0.29
Fe	0.20	0.17
Cl	1.62	0.05
Na	0.16	0.01
Zn	0.008	0.006
Mn	0.070	0.043
Cu	0.002	0.002

[1]**Shammout and Zakaria b (2015)**

Table 1b. Analysis of water in the presence of duckweed (*Lemna gibba*)[1]

Parameter	Water analysis in mg/l	Allowed limits (mg/l)[2]
Ca	94	230
Mg	28	100
K	15	-
Cl	330	400
SO4	162	500
PO4	4.60	30
Na	203	230
NO3	21	70
Zn	<0.02	5
Pb	<0.01	0.20
Cd	<0.002	0.01
Cu	<0.01	0.20

[1] Shammout and Zakaria (a,b, 2015), [2]Jordan standards 893, 2006

Table 2. % Diet composition and content of dietary treatments

Ingredient	Control	T1(10% of the Soybean meal)	T2(20% of the Soybean meal)
------------------------54-63wks------------------------			
------------------------%------------------------			
Corn	66.00	66.00	66.00
Soybean meal (47.6% CP)	22.00	19.80	17.60
Limestone (ground)	9.20	9.20	9.20
Dicalcium Phosphate	0.30	0.30	0.30
Concentrate [1]	2.50	2.50	2.50
Duckweed (*Lemna gibba)*	0.00	2.20	4.40
Nutrient Composition			
ME (Kcal/Kg)	2716.60	2730.30	2744.01
Crude Protein	15.73	15.29	14.86
DL-methionine	0.41	0.39	0.39
Lysine	0.87	0.89	0.93
Threonine	0.61	0.58	0.55
Tryptophan	0.20	0.19	0.17
Ether Extract	2.86	2.92	2.98
Crude Fiber	2.55	2.83	3.11
Ca	4.04	4.16	4.28
P- nonphytate	0.39	0.40	0.41
Na	0.17	0.17	0.17
Choline Chloride mg/kg	40.00	40.00	40.00
Cost price(JD)/ ton[2]	243.42	237.92	232.42

[1]2.5% Layers concentrate contains: 0.3%NaCl, 400,000IU vitamin A, 800,000 IU vitamin D3, 800mg/kg vitamin E, 140 mg/kg vitamin K, 24mg/kg vitamin B1, 200mg/kg vitaminB2, 280mg/kg pantothenic acid, 1000mg/kg niacin, 72 mg/kg vitamin B6, 800 B12, 20 mg/kg folic acid, 2000 mg/kg biotin, 4000mg/kg vitamin C, Fe as sulfate 1760 mg/kg, 200mg/kg Cu as sulfate, 2000mg/kg Zn as sulfate, 2480mg/kg Mn as oxide, 52 mg/kg I as potassium, 9mg/kg Se as selenite, 260 mg/kg antioxidant, 2000mg/kg enzyme.

[2] Cost price according to metric ton cost of feed in Jordanian dinars at the time the trial was carried**.**

Table 3. Performance of laying hens fed diets containing 0%, 10%, or 20% duckweed

		Duckweed Inclusion Rate (%)				
Period	**Parameter Measured**	**DW0%[1]**	**DW10%[1]**	**DW20%[1]**	**SEM[2]**	**P-Value**
1 – 3 wk	Feed Intake (g/hen/day)	124.24[a]	123.94[a]	96.63[b]	5.40	**
	Feed Conversion (g/g)[3]	1.74	1.71	1.57	0.08	NS
	Bodyweight (g/bird)	1767.12	1711.33	1677.67	48.72	NS

	Egg Laying Rate (%)	87.76[a]	87.16[a]	75.46[b]	3.41	*
	Egg Weight (g)	63.15	63.81	62.14	1.06	NS
	Egg Mass (g)[4]	54.82[a]	56.18[a]	46.91[b]	2.49	*
	Mortality Rate (%)[5]	1.39	1.39	1.39	1.35	NS
4 – 6 wk	Feed Intake (g/hen/day)	123.57[a]	116.72[a]	101.66[b]	6.30	*
	Feed Conversion (g/g)	1.88	1.78	1.42	0.14	NS
	Bodyweight (g/bird)	1770.82	1711.68	1663.49	47.71	NS
	Egg Laying Rate (%)	85.74[a]	82.93[a]	64.27[b]	5.83	*
	Egg Weight (g)	64.82	63.43	63.02	0.84	NS
	Egg Mass (g)	55.71[a]	52.92[a]	40.38[b]	4.01	*
	Mortality Rate (%)	0.19	4.16	6.94	3.02	NS
7 – 9 wk	Feed Intake (g/hen/day)	126.09[a]	117.02[a]	88.10[b]	6.48	**
	Feed Conversion (g/g)	1.74	1.78	1.90	0.12	NS
	Bodyweight (g/bird)	1761.20	1696.37	1645.37	49.15	NS
	Egg Laying Rate (%)	83.69[a]	76.34[a]	55.25[b]	5.36	= 0.001
	Egg Weight (g)	64.15	62.77	66.14	1.32	NS
	Egg Mass (g)	53.69[a]	47.96[a]	36.72[b]	3.78	*
	Mortality Rate (%)	1.39[b]	2.77[b]	16.67[a]	3.53	*
1 – 9 wk	Feed Intake (g/hen/day)	124.24[a]	119.30[a]	96.24[b]	4.97	*
	Feed Conversion (g/g)	1.78	1.75	1.64	0.08	NS
	Bodyweight (g/bird)	1765.34	1705.88	1664.25	46.44	NS
	Egg Laying Rate (%)	85.63[a]	82.32[a]	64.91[b]	3.98	**
	Egg Weight (g)	64.01	63.27	63.85	0.90	NS
	Egg Mass (g)	54.75[a]	52.23[a]	41.46[b]	2.84	= 0.01
	Mortality Rate (%)	1.03	2.04	7.11	1.94	NS

[a-b] Means within rows with varying superscripts differ significantly * ($P<0.05$, ** $P<0.01$)

[1] Dietary treatments used in the trial: DW0% (Control with 0% duckweed); DW10% (duckweed inclusion rate at 10%); DW20% (duckweed inclusion rate at 20%)

[2]SEM: standard error of the mean

[3]Feed Conversion Ratio (g feed intake: g dozen eggs).

[4]Egg Mass = Egg Laying Rate x Egg Weight (g)

[5]Mortality Rate corrected for both feed intake and feed conversion ratio

Table 4. Egg composition of laying hens fed diets containing 0%, 10%, or 20% duckweed

		Duckweed Inclusion Rate (%)				
Period	**Parameter Measured**	**DW0%[1]**	**DW10%[1]**	**DW20%[1]**	**SEM[2]**	**P-Value**
1 – 3 wk	Wet Shell Percent (%)	14.22	14.14	14.08	0.310	NS
	Dry Shell Percent (%)	9.73	9.70	9.68	0.132	NS
	Wet Albumen Percent (%)	51.62	51.64	50.56	0.773	NS
	Dry Albumen Percent (%)	4.02	3.91	3.86	0.085	NS
	Wet Yolk Percent (%)	28.15	28.02	28.51	0.382	NS
	Dry Yolk Percent (%)	14.73	14.77	14.69	0.239	NS
4 – 6 wk	Wet Shell Percent (%)	14.03	14.30	13.76	0.289	NS
	Dry Shell Percent (%)	9.76	9.87	9.60	0.146	NS
	Wet Albumen Percent (%)	52.49	51.22	51.92	0.770	NS
	Dry Albumen Percent (%)	3.89	3.70	3.83	0.089	NS
	Wet Yolk Percent (%)	29.13	29.10	28.91	0.438	NS
	Dry Yolk Percent (%)	15.75	15.42	15.47	0.299	NS
7 – 9 wk	Wet Shell Percent (%)	14.54	14.36	14.30	0.337	NS
	Dry Shell Percent (g)	9.55	9.52	9.43	0.135	NS
	Wet Albumen Percent (%)	53.89	51.86	53.12	0.691	NS
	Dry Albumen Percent (%)	4.12[a]	3.79[b]	3.96[ab]	0.091	*
	Wet Yolk Percent (%)	28.37	29.26	28.99	0.410	NS
	Dry Yolk Percent (%))	14.98	15.54	15.24	0.326	NS
1 – 9 wk	Wet Shell Percent (%)	14.26	14.26	14.06	0.250	NS
	Dry Shell Percent (%)	9.69	9.69	9.57	0.101	NS
	Wet Albumen Percent (%)	52.67	51.48	51.80	0.583	NS
	Dry Albumen Percent (%)	4.02[a]	3.77[b]	3.89[ab]	0.063	*
	Wet Yolk Percent (%)	28.61	28.68	28.88	0.270	NS
	Dry Yolk Percent (%)	15.07	15.29	15.17	0.184	NS

[a-c] Means within rows with varying superscripts differ significantly* (P <0.05)

[1] Dietary treatments used in the trial: DW0% (Control with 0% duckweed); DW10% (duckweed inclusion rate at 10%); DW20% (duckweed inclusion rate at 20%).[2]SEM: standard error of the mean.

Table 5. Egg quality parameters of laying hens fed diets containing 0%, 10%, or 20% duckweed

		Duckweed Inclusion Rate (%)				
Period	**Parameter Measured**	**DW0%[1]**	**DW10%[1]**	**DW20%[1]**	**SEM[2]**	**P-Value**
1 – 3 wk	**Haugh Units**	77.61	79.54	82.85	2.51	NS
	Yolk Color	4.75[c]	5.39[b]	5.80[a]	0.138	= 0.0001
	Shell Thickness (mm)	0.338	0.330	0.331	0.0051	NS
	Blood Spots Percent (%)	1.03[b]	1.99[b]	12.10[a]	3.95	*
	Cracked Egg Percent (%)	1.03	0.77	2.08	1.32	NS
	Grade AA Egg Percent (%)	85.91	83.33	81.15	9.05	NS
	Grade A Egg Percent (%)	8.23	14.34	16.32	7.92	NS
	Grade B Egg Percent (%)	8.33	11.11	2.76	5.46	NS
4 – 6 wk	**Haugh Units**	85.04	84.01	79.37	2.61	NS
	Yolk Color	5.82[b]	6.70[b]	6.91[a]	0.175	= 0.0001
	Shell Thickness (mm)	0.349	0.352	0.342	0.0047	NS
	Blood Spots Percent (%)	1.18	0.65	2.57	0.861	NS
	Cracked Egg Percent (%)	1.40	1.64	2.31	0.828	NS
	Grade AA Egg Percent (%)	97.22	88.88	77.78	6.45	NS
	Grade A Egg Percent (%)	2.78	5.56	11.11	4.63	NS
	Grade B Egg Percent (%)	1.63	3.92	11.11	2.98	NS
7 – 9 wk	**Haugh Units**	86.20	86.67	82.93	2.01	NS
	Yolk Color	6.52[c]	7.20[b]	7.86[a]	0.160	= 0.0001
	Shell Thickness (mm)	0.342	0.344	0.343	0.0055	NS
	Blood Spots Percent (%)	0.25	0.53	3.76	1.11	NS
	Cracked Egg Percent (%)	0.96	1.32	1.74	0.693	NS
	Grade AA Egg Percent (%)	88.89	91.66	83.33	6.46	NS
	Grade A Egg Percent (%)	5.64	6.81	4.21	3.98	NS
	Grade B Egg Percent (%)	6.34	4.04	11.84	4.59	NS
1 – 9 wk	**Haugh Units**	82.60	83.83	81.72	1.47	NS
	Yolk Color	5.71[c]	6.41[b]	6.86[a]	0.094	= 0.0001
	Shell Thickness (mm)	0.343	0.343	0.339	0.0032	NS
	Blood Spots Percent (%)	0.75[b]	1.15[b]	6.13[a]	1.52	*
	Cracked Egg Percent (%)	1.13	1.26	2.03	0.765	NS
	Grade AA Egg Percent (%)	87.94	87.87	80.66	5.89	NS
	Grade A Egg Percent (%)	5.49	8.67	10.85	3.21	NS
	Grade B Egg Percent (%)	5.22	5.90	9.25	2.60	NS

[a-c] Means within rows with varying superscripts differ significantly * (P <0.05), (P=0.0001*)*

[1] Dietary treatments used in the trial: DW0% (Control with 0% duckweed); DW10% (duckweed inclusion rate at 10%); DW20% (duckweed inclusion rate at 20%)

[2]SEM: standard error of the mean.