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ENHANCING ANTIOXIDANT STATUS, PRODUCTIVE AND REPRODUCTIVE PERFORMANCE FOR POST-MOLT BROILER BREEDERS BY USING MACA POWDER (*LEPIDIUM MEYENII*)

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Abstract

This study was designed to evaluate the effects of using Maca powder (*Lepidium meyenii*) in the diet on egg production traits, fertility, hatching characteristics, and some blood parameters for broiler breeders (Arbor-Acres Plus) post-molting. A total of 180 Females and 15 Males, 62 weeks old, Arbor Acres Plus broiler breeders were induced to forced molting with ZnO in diet with a moderate decrease in lighting schedule from 16 to 8 h and feed restriction at 50 g/bird daily. Post-molting birds were randomly divided into three groups according to Maca powder as follows: the 1st group (M0) was fed the basal diet without Maca, while the 2nd (M1) and the 3rd (M2) groups were fed the basal diets supplemented with Maca powder at the levels of 1 and 2g Maca/kg diet, respectively. All groups were replicated into five replicates of 13 birds each (1 male + 12 females). The trial lasted for 12 weeks. According to the present study, the laying rate was significantly ($P<0.05$) increased by using Maca powder at different levels, without affecting egg weight and egg mass. Furthermore, the fertility rate and hatchability of settable eggs were significantly ($P<0.05$) increased by using Maca powder at 1 and 2g /kg diet. Additional results showed that using Maca powder significantly ($P<0.05$) decreased early embryonic mortality rate compared with control. Data indicated that Maca had no significance on the hatchability of fertile eggs, chick weight, and late embryonic mortality rate. The results of blood biochemical parameters revealed that serum cholesterol was significantly ($P<0.05$) reduced by using Maca powder. Data indicated

that TSD and TAOC were significantly ($P<0.05$) higher in the M1 and M2 groups compared with control. It could be concluded that Maca powder (*Lepidium meyenii*) administration in diets for post-molt broiler breeders at levels of 1 and 2g /kg could be improved productive and reproductive traits also, enhance antioxidant status.

Keywords: Maca powder, broiler breeders, performance, antioxidant status, molting.

Introduction

Poultry production has gone through a lengthy process of progress and growth that transformed it from a simple rudimentary industry to an integrated one. In this industry, there is also international competitiveness, and cutting-edge scientific techniques and modern technology are used. According to [1], forced molting is an effective method for increasing the production capacity of an existing flock of layer chickens and is particularly cost-effective for developing nations. That may be because it lowers production costs in comparison to replacing the flock with younger ones [2]. After the force molting procedure used on Hubbard flex cocks at 65 weeks of age, **Hassan *et al.*** [3] showed a remarkable decrease in fertility and semen quality attributes. *Lepidium meyenii*, also known as Maca, has been planted and utilized for food, medicine, and to increase reproduction in both people and animals [4-6]**.** It has been referred to as a "lost crop of the Incas" [7].

Maca is one of the most medicinal plants rich in antioxidants, and vitamins, and it is also very rich in flavonoids that protect against many diseases [8]. According to **Qiu *et al.*** [9] Maca roots are utilized for a variety of medical purposes, including as an antioxidant and as a significant immune system stimulant [10]. By its impact on sex hormones and their receptors, it impacts sexual potency and boosts fertility [11]. **Chacon** [12] stated that Maca caused female mice to have more follicles. Maca has many bioactive compounds such as N-benzyl-palmitamide, benzylisothiocyanate, glucosinolates, and phenolics [13-15]. According to studies by **Cheng *et al.*** [16] and **Melnikovova *et al.*** [17], Maca enhances fertility and sexual function without altering levels of reproductive hormones. It also controls health through its anti-osteoporosis and fatigue-reducing qualities [18]. Moreover, studies have demonstrated that Maca reduces plasma fatty acid concentrations [19].

Only a few studies have been carried out to evaluate the effects of Maca (*Lepidium meyenii*) on broiler breeders' diet post-molting. Therefore, the current study aimed to determine how using Maca powder (*Lepidium meyenii*) to broiler breeders' diets post-molting affects their laying performance, fertility, hatching characteristics, and some blood parameters.

MATERIAL AND METHODS

This experiment was carried out at a commercial farm in Minia governorate, Egypt. It was designed to study the effects of using Maca powder (*Lepidium meyenii*) in diets for broiler breeders (Arbor-Acres Plus) post-molting during the period from December 2022 to March 2023.

Experimental design:

A total number of 180 Females and 15 Males, 62 weeks old, Arbor Acres Plus broiler breeders were induced to forced molting with ZnO in the diet at the level of 3000 mg/kg with a moderate decrease in lighting schedule from 16 to 8 h and feed restriction at 50 g/bird daily. The molting phase continued for 6 weeks until a 27% reduction in their body weight was achieved. After completion of molting, birds were randomly distributed into three groups according to Maca addition as follows: the 1st group (M0) was fed the basal diet without Maca, while the 2nd (M1), the 3rd (M2) groups were fed the basal diets supplemented with Maca at the levels of 1 and 2g Maca/kg diet, respectively. All groups were replicated into five replicates of 13 birds each (1 male + 12 females). The trial lasted for 12 weeks.

Housing the experimental birds:

The birds were all accommodated within floor pens of identical dimensions, situated in a unified space within a closed environment. These deep litter floor pens measured (100 cm L× 200 cm W× 150 cm H) and were maintained under consistent hygienic and environmental conditions, facilitated by automated ventilation. Throughout the experimental phase, the birds were subjected to a uniform lighting regimen of 15 hours of light followed by 9 hours of darkness per day. This illumination was achieved through the utilization of 60-watt incandescent lamps suspended at a height of 2 meters from the floor. All pens were provided with electrical heaters without fans that provided the inside temperature within a comfortable range (22°C) and the relative humidity ranged between 55 and 65%. Two separate-sex feeders, a drinker, and a nest box were used in each pen.

Experimental diets:

All experimental birds were fed on a basal commercial diet. A basal diet was formulated to meet all nutrient requirements according to **NRC (1994)** [20]. The basal diet for males included 12.07% crude protein, 6.10% crude fiber, 3.39% fat, 0.95% calcium, 0.37% available phosphorus, and 11.29MJ/kg metabolic energy. The basal diet for females included 13.30% crude protein, 3.16% crude fiber, 2.96% fat, 3.51% calcium, 0.39% available phosphorus, and 11.69MJ/kg metabolic energy. Female birds were limited to approximately 173g of feed per day, whereas males were restricted to around 162g per day. The dry Maca composition analysis revealed a content of 10.2% crude protein, 59.0% carbohydrates, 2.20% fat, and 8.5% fibers [21].

Parameters studied:

1- Egg production:

The eggs were daily collected two times at 9 AM and 4 PM to calculate egg numbers. **Laying rate (%)** = (Egg number/ Hen number) × 100. Eggs were weighed individually and recorded every

day to calculate average egg weight **(g)** and egg mass **(g)**. **Egg mass** = (Egg production × Egg weight) / 100.

2- Fertility rate and hatching traits:

During the final six weeks of the experiment, 35 eggs were gathered from each replicate and subsequently stored for 7 days at temperatures ranging between 15-18ºC and relative humidity levels of 70-75% before incubation. Incubation of all eggs across treatments adhered to standardized conditions: temperatures set at 37.8°C with relative humidity maintained at 55-60% for days 1 to 18 of incubation and adjusted to 36.8°C with relative humidity ranging between 60-65% during the last three days of incubation. Utilizing an automatic Paterzime incubator, the incubation process was executed. On the seventh and eighteenth day of incubation, eggs were candled to identify infertile eggs and those with early or late-stage dead embryos. Following the incubation period, unhatched eggs were carefully examined to precisely determine fertility rates, hatchability of settable and fertile eggs, as well as early and late embryonic mortality percentages. Post-hatch, chicks were weighed using small-scale instruments (in grams).

3- Hematological Studies:

Upon conclusion of the experiment, three female birds (3♀) per replicate were randomly selected, and blood samples were drawn from each bird into both heparinized and unheparinized tubes. The heparinized tube contained whole blood, utilized for assessing the blood's physical properties. Meanwhile, the blood in the other tubes was centrifuged at 3000 rpm for 15 minutes to separate the blood plasma. The obtained plasma was then preserved at -80°C until further biochemical and immunological analyses could be conducted.

a. Hematocrit (PCV) and Hemoglobin (Hb):

To ascertain the Hematocrit value (PCV), two measurements were conducted for each sample using the microhematocrit technique as follows: Two heparinized capillary tubes were filled with blood, their ends securely sealed, and then subjected to centrifugation in a microcapillary centrifuge for ten minutes at 1200 rpm. The PCV measurements were determined by a circular reader [22].

Blood hemoglobin percentage was estimated according to the recommendation of the international committee for standardization in hematology [23] using reagent kits purchased from Randox Company (United Kingdom).

b. Biochemical Determinations:

Plasma total protein levels were assessed following the method outlined by **Doumas et al.** [24], while albumin concentrations were determined using the protocol described by **Doumas et al.** [25], both utilizing assay kits provided by BioMed Chemical Company, Egypt. Globulin levels were calculated by subtracting the albumin values from the total protein values. Plasma glucose levels were measured according to **Trinder** [26] with assay kits from Diamond Chemical Company, Germany. Plasma cholesterol levels were determined following the procedure outlined by **Watson** [27] using assay kits from BioMed Chemical Company, Egypt. Serum levels of aspartate aminotransferase (AST) and alanine transaminase (ALT) were determined as per the method detailed by **Reitman and Frankel** [28], with assay kits provided by BioMed Chemical

Company, Egypt.

c. Antioxidative status

The level of malondialdehyde (MDA) in the blood was determined following the protocol outlined by **Placer et al.** [29]. The blood's total antioxidant capacity (TAOC) was evaluated using the calorimetric method described by **Koracevic et al.** [30]. The measurement of serum total superoxide dismutase (TSOD) was conducted according to the method established by **Misra and Fridovich** [31].

Statistical analysis:

Data were statistically analyzed by the completely randomized design using the general linear model's procedure of **SAS (1998)** [32]. All data obtained were analyzed by using the following Model:

$$Yij = \mu + Ti + Eij$$

Where, Yij = an observation; μ = general mean; Ti = fixed effect of i^{th} Maca administration, i= 1 and 2 and 3 (0, 1 and 2g Maca powder/kg); Eij = error of the model, which included all the other effects not specified in the mixed model.

Differences among means of the experimental groups were testified for significance by Duncan's multiple range test [33] with a 5% level of probability.

RESULTS AND DISCUSSION

1- Egg production:

Data in Table (1) show that LR% was significantly higher ($P<0.05$) for birds who received Maca powder in their diets at levels of 1 and 2g/kg diet compared with the control. Data reported no significant effects in LR% between M1 and M2 groups. On the other hand, Maca powder at different levels did not have any significant effect on egg weight (g) and egg mass (g/b/d) for birds post-molting.

It is well known that antioxidants derived from plants enhance reproductive performance. The Maca plant contains alkaloids that greatly stimulate ovarian follicles [34]. According to **Nadia *et al.*** [35], adding herbs to laying hens as natural antioxidants can increase the laying rate.

Our results are in agreement with **Osfor** [36], who stated that using Ginseng plant, which has similar antioxidant effects to Maca, in diets for Japanese quails at levels of 2 and 4 mg/kg significantly ($P<0.05$) increased egg production. Similarly, **Jang *et al.,*** [37] reported that the addition of fermented ginseng by-product to laying hens' diets increased egg weight and yield. Additionally, **Korkmaz *et al.*** [38], showed that adding Maca powder at 5 and 10g/kg diet had no significant effects on egg weight for laying hens in the post-peak period (from 56 to 72 weeks of age). Also, **Gul *et al.,*** [39] revealed that egg weight and mass were not significantly affected by adding Maca powder at 1 and 2g/kg in diets for laying quails.

In contrast**, Korkmaz *et al.*** [38], showed that adding Maca powder at 5 and 10g/kg diet had no significant effects on egg production for laying hens in the post-peak period. Additionally, **Gul et al.** [39] demonstrated that the inclusion of Maca powder at 1 and 2g/kg in laying quail diets did not significantly impact egg production.

Table (1): Effect of Maca powder on egg production traits for broiler breeders post molting.

Treatment / Trait	M0	M1	M2	SEM	P-value
Laying rate %	63.86[b]	66.33[a]	65.77[a]	0.54	0.0214
Egg weight (g)	68.37	68.24	68.45	0.50	0.9556
Egg mass (g/b/d)	43.66	45.26	45.03	0.57	0.1470

[a] and [b] Mean with different superscripts in the same raw are significantly different ($P< 0.05$).

M0= Basal diet without Maca (control), **M1**= 1g Maca/ kg diet, **M2**= 2g Maca/kg diet, **SEM=** Standard error mean

2- Fertility rate and hatching traits:

Results in Table (2) revealed that fertility rate and hatchability of settable eggs % were significantly ($P<0.05$) higher in M1 and M2 groups compared with control (M0), with no significant differences between M1 and M2 groups. Data showed that early embryonic mortality rate % was significantly ($P<0.05$) decreased for birds who received Maca in their diets at levels 1 and 2g/kg as compared to in the control group. Nevertheless, the findings indicated that the hatchability of fertile eggs, late embryonic mortality rate, total embryonic mortality rate percentages, and chick weight at hatching remained unaffected by the addition of Maca powder to diets at 1 and 2g/kg following molting.

As stated by **Gan *et al.*,** [40], the most important substances for the antioxidation of Maca are alkaloids and phenols. **Kurt** [41], suggested that antioxidants may work by enhancing LH levels to increase fertility. According to **Turgud and Narinc,** [34], using antioxidants derived from plants improves reproductive characteristics like sperm, semen, and oocyte quality. Also, **Yucra *et al.*** [42], revealed that Maca regulates sperm count by maintaining the balance between oxidant and antioxidant status. Maca has been linked to improved sperm parameters, enhanced androgen-like effects, and improved sexual behavior, according to a number of in vivo studies [43-49]. It was determined that improving the diets of breeder turkey hens by adding plant seeds and root extracts with antioxidant properties enhances the hens' fertility, hatchability, and embryonic viability [50-55]

The present study is in agreement with **Azazi *et al.*** [56], who found that semen quality, fertility, and hatchability rate were significantly improved in layer breeder-fed diets supplemented with ginseng, which has similar antioxidant effects to Maca. Moreover, **Uchiyama *et al.*,** [57]

indicated that the use of Maca improves fertility. As well as **Turgud and Narinc** [34] illustrated that the total embryonic mortality was significantly (P<0.05) decreased by using Maca powder at the level of 0.1% in diets for Japanese quails, however early or late embryonic mortality and chick weight at hatching were not significantly affected by using Maca powder at levels of 0.05 or 0.1% in diets for Japanese quails. Also, **Fouda *et al.*** [58], showed that Muscovy ducks fed diets supplemented with Maca at the level of 500mg/kg had the highest (P<0.05) fertility rate as compared to control during the period of 34 to 51 weeks of age.

Table (2): Effect of Maca powder on fertility and hatching traits for broiler breeders post molting.

Trait \ Treatment	M0	M1	M2	SEM	P-value
Fertility rate %	91.35[b]	92.72[a]	92.96[a]	0.34	0.0145
Hatchability of settable eggs %	84.16[b]	86.05[a]	86.03[a]	0.31	0.0023
Hatchability of fertile eggs %	92.14	92.80	92.54	0.30	0.3399
Chick weight (g)	46.23	46.34	46.29	0.49	0.9896
Early embryonic mortality rate %	3.38[a]	2.56[b]	2.50[b]	0.23	0.0400
Late embryonic mortality rate %	3.81	4.12	4.43	0.26	0.2974
Total embryonic mortality rate %	7.18	6.68	6.93	0.29	0.4949

[a] and [b] Means with different superscripts in the same row are significantly different (P< 0.05).

M0= Basal diet without Maca (control), **M1**= 1g Maca/ kg diet, **M2**= 2g Maca/kg diet, **SEM=** Standard error mean

3- Blood parameters:

As shown in Table (3), it could be noted that cholesterol level was significantly (P<0.05) reduced for birds received Maca in diets as compared to those in control group. While data showed that total superoxide dismutase and total antioxidant capacity levels were significant (P<0.05) higher in M1 and M2 groups compared with control one. Results indicated that there were not significant differences (P>0.05) between birds in M1 and M2 groups in cholesterol, TSOD and TAOC levels. Our findings illustrated that Maca powder at levels 1 and 2g/kg diets for broiler breeders post molting had not significant effects on total protein, albumen, globulin, albumen/ globulin ratio, glucose, package cell volume, hemoglobin, alanine transaminase, aspartate transaminase and malondialdehyde levels.

The improvements in antioxidant status for birds that received Maca powder in their diets may be attributed to the fact that Maca contains several compounds with antioxidant activity, such as phenols, alkaloids, glutathione peroxidase, and glucosinolates [14, 59]. According to **Sandoval *et al.*,** [60], the anti-oxidative activity of Maca is perhaps a result of aromatic isothiocyanates.

Additionally, **Vecera *et al.,*** [13] reported that rats fed diets supplemented with Maca at 1% had the highest (P<0.05) total superoxide dismutase activity in the liver compared with control.

The cause of the drop in cholesterol concentration in groups that received Maca powder could be because of the Maca plant's function as a natural antioxidant, which eliminates free radicals from the body before they reach the reaction chin; this lowers the rancidity of fat and improves the body's antioxidant levels [61]. **Al-Allaf and Al- Kennany** [62], illustrated that the decline in cholesterol levels might be caused by the presence of phenols in Maca roots, which are important antioxidants that decrease the absorption of cholesterol while increasing the secretion of bile salts. According to **Tafuri *et al.*** [11], the reduction in cholesterol concentration may be because of the high content of vitamin C found in Maca roots, which is known to have a significant effect on lowering cholesterol levels by speeding up thyroid activity and thyroxin secretion.

Our observations in harmony with **Korkmaz *et al.*** [38]**,** who stated that using Maca powder significantly (P<0.05) improved antioxidant status in laying hens when added in their diets at levels of 5 and 10 g/kg in the post-peak period. Also, **Fouda *et al.*** [58], showed that the adding of Maca at level of 500mg/kg diet of Muscovy ducks significantly (P<0.05) improved total superoxide dismutase and total antioxidant capacity levels in seminal plasma. Furthermore, **Gul, *et al.*** [39] stated that using Maca powder in diet for laying quails at level of 2g/kg significantly (P<0.05) reduced serum cholesterol, while Maca at levels of 1 or 2g/kg had not significant effects on serum glucose and total protein. The same results were observed for Ross 308 breeder males by adding of Maca tuber powder at levels of 0.5, 1.0, and 1.5g/kg diet [63]**,** who added that Maca significantly improved antioxidant status. Also, **Ragab *et al.*** [64], showed that oral administration of Maca extract for the growing V-line 6-week-old buck rabbits at levels 400 mg/head twice per week significantly (P<0.05) improved total antioxidant capacity and superoxide dismutase compared with the control group.

In contrast, **Korkmaz *et al.*** [38], observed no significant differences in serum cholesterol concentrations in laying hens when fed diets supplemented with Maca powder at levels of 0, 5, and 10 g/kg compared. Additionally, the same observation was noted in rats [65]**.** As well as **Ragab *et al.*** [64], showed that hemoglobin and hematocrit were significantly (P<0.05) increased, while alanine transaminase, malondialdehyde, glucose, and total cholesterol levels significantly (P<0.05) reduced in the growing V-line 6-week-old buck rabbits which orally administered Maca **(***Lepidium meyenii***)** extract at levels 400 mg/head twice per week compared with the control group [66-68].

Table (3): Effect of Maca powder on some blood parameters for broiler breeders post molting.

Trait \ Treatment	M0	M1	M2	SEM	P-value
Total protein (g/dl)	6.13	6.11	6.10	0.20	0.8943
Albumen (g/dl)	2.05	2.07	2.07	0.14	0.9770
Globulin (g/dl)	4.09	4.05	4.03	0.15	0.8974
Albumen/ Globulin ratio (g/dl)	0.50	0.52	0.52	0.04	0.9421
Glucose (mg/dl)	220.00	220.06	220.35	1.43	0.9831
Cholesterol (mg/dl)	220.59[a]	169.06[b]	194.36[b]	2.48	<0.0001
Package cell volume %	31.37	31.31	31.51	0.39	0.9318
Hemoglobin (g/dl)	9.17	9.16	9.16	0.07	0.9946
Alanine Transaminase (IU/L)	9.75	9.76	9.73	0.12	0.9839
Aspartate transaminase (IU/L)	188.39	188.47	188.31	1.42	0.9968
Total superoxide dismutase (U/mL)	375.32[b]	401.45[a]	402.75[a]	2.32	<0.0001
Malondialdehyde (nmol/mL)	7.93	7.84	7.78	0.10	0.4953
Total antioxidant capacity (mmol/mL)	1.52[b]	1.75[a]	1.81[a]	0.02	<0.0001

[a] and [b] Means with different superscripts in the same row are significantly different (P< 0.05).
M0= Basal diet without Maca (control), **M1**= 1g Maca/ kg diet , **M2**= 2g Maca/kg diet**SEM=** Standard error mean

Conclusion

This research demonstrates that dietary inclusion of Maca powder at levels of 1 and 2 grams per kilogram of diet significantly enhances the laying rate, fertility rate, and hatchability of settable eggs without affecting the egg weight and egg mass. Moreover, the supplementation of Maca powder has been shown to decrease early embryonic mortality rates, although it does not significantly impact the hatchability of fertile eggs, late embryonic mortality rates, or chick weight at hatching.

The biochemical parameters studied reveal a notable reduction in serum cholesterol levels and an increase in total superoxide dismutase (TSOD) and total antioxidant capacity (TAOC) in groups receiving Maca powder, indicating an improved antioxidant status. These findings suggest that Maca powder possesses bioactive compounds that contribute to its antioxidative properties, which in turn may enhance the reproductive performance and overall health of the birds.

The research is methodologically sound, employing a well-structured experimental design, appropriate statistical analysis, and clear presentation of results. The study's findings are

significant as they suggest that Maca powder could be a valuable dietary supplement for improving the productive and reproductive traits of broiler breeders post-molting, as well as enhancing their antioxidant status. This could have practical implications for the poultry industry, potentially leading to more cost-effective and sustainable production practices.

In conclusion, the administration of Maca powder in the diets of post-molt broiler breeders at the investigated levels appears to be a promising strategy to improve their performance and health. Future research could explore the long-term effects of Maca supplementation and its potential benefits in different poultry breeds and production systems.

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