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Alleviation of the V-Line rabbit bunnies' weaning stress by *Moringa Oleifera* incorporation with feed

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ABSTRACT

Raising rabbits has vital sustainable development features, as a sustainable development system of meat production strongly relies on its efficacy. The development system of meat production is correlated with the animals' capability for converting feed to meat. Weaning possess a marked stress on rabbits, because of lack of their normal caecal microbiota, hence young rabbits became more vulnerable to diseases. Numerous antibiotics alternatives have been successfully utilized, such as herbal medicines as natural supplements in animal feed. The leaves of *Moringa Oleifera* could potentially be utilized as a growth promoter due to their antimicrobial activity, antioxidant properties, hepato-protective effect, preservation of renal function, and hypocholesterolemic activity. Twelve recently weaned V-Line rabbit bunnies about four weeks of age and average body weight of 0.74±0.07 were separated equally to feed either normal-prepared rabbit feed (Control group (C); n=6) or moringa-prepared feed (*Moringa Oleifera* group (MO); n=6) for 4 weeks. Performance (feed intake, body weight, and weight gain), behavior, and serum biochemical analysis (oxidative stress, liver, and kidney function) were assessed. Results revealed that the incorporation of MO in recently weaned rabbit feed enhanced their performance and behavior, as well as lipid peroxidation and glutathione didn't differ significantly and preserved liver and kidney function. In conclusion, MO plays a crucial role in weaning stress alleviation via their antioxidant, hepatic and renal function preservation and their positive effects on rabbit bunnies' performance and behavior. Hence, the data suggests the use of MO leaves as a feed additive for rabbit bunnies to alleviate weaning stress.

Introduction

Rabbits (*Oryctolaus cuniculus*) are small pseudo-ruminant species kept for the fiber wool, highly nutritious proteins, and reduced-fat content, along with their purposes related to research in medicine (Hamid *et al*., 2019; Elbarbary *et al*., 2023). Raising rabbits has vital sustainable development features. The development system of meat production strongly relies on its efficacy, which is correlated with the animals' capability for converting feed to meat, and the efficiency of protein conversion into meat is superior in rabbits than in other animals, in being able to convert 20% of the protein consumed in their food supply into lean muscle (Cesari *et al*., 2018; Siddiqui *et al*., 2023).

Weaning is challenging for rabbits as their normal caecal microbiota are still lacking and young rabbits are more vulnerable to diseases. Earlier weaning increased the vulnerability to bacterial enteritis, while maternal caecotrophs' consumption promotes the caecum population maintaining a healthy gut flora. In addition, the levels of ammonia in the caecum reduced after weaning because of the change feeding from milk to solids which is attributed to the acidic pH of the caecum as the volatile fatty acid levels rise (Varga, 2014; Kazana *et al*., 2022). Post-weaning, rabbits get upset frequently by shifts in house and nutrition, transportation, and interaction with others. Utilizing a small room with many animals boosts the probability of infection transmission, the presence of pathogenic bacterial and viral strains, and the change in diet could trigger the development of soft caecotrophs that are not ingested increasing their vulnerability to diseases (Varga, 2014).

The efficiency of antibiotics in boosting animal growth performance resulted in their widespread application as growth promoters. Concerns about their toxicity, consequences over time, and bacterial resistance to some antibiotics, antibiotics have been prohibited in the European Union as promoters of growth since 2006 because of the adverse impacts they have on the well-being of humans (Mc Carteny, 2002). Because of this, numerous antibiotics alternatives have been successfully utilized, such as herbal medicines as natural supplements in animal feed (Saki *et al*., 2012).

Drumstick tree\ miracle tree\Horseradish tree or *Moringa Oleifera* is originally from Northern India, but now it has been found in abundance over the Americas, Africa, Europe, Oceania, and Asia (Palada, 1996; Fuglie, 2001). The leaves of *Moringa Oleifera* could potentially be used as a growth promoter due to its antimicrobial activity (Caceres *et al*., 1990; Suarez *et al*., 2005), antioxidant properties (Sreelatha and Padma, 2009; Moyo *et al*., 2012), hepato-protective effect (Pari and Kumar, 2002; Samiksha *et al*., 2023), preserve renal function (Ouédraogo *et al*., 2013), and hypocholesterolemic activity (Pari and Kumar, 2002; Slimani *et al*., 2007). The leaves of the *Moringa Oleifera* plant are a rich source of over ninety nutrient substances, involving lipids, carbohydrates, protein, along with fiber. They are also an excellent source of vitamin A and vitamins (B-complex, C, D, and K), in addition to certain essential macro- and micro-elements that include potassium, calcium, magnesium, zinc, copper, iron, and selenium (El-Badawi *et al*., 2016; Brilhante *et al*., 2017). Besides, its usefulness as a source of nutrition can be seen by the substantial nutritional value of the dried leaves (Moyo *et al*., 2011).

By employing medicinal plant leaves and extracts as nutritional supplements helps improve animal well-being and performance (Mahmood *et al*., 2010). Wagner *et al*. (2008) mentioned that a method of feeding

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rabbits that satisfies all their dietary requirements and provides most of the nutrients they need, allows rabbits inhabiting these types of enclosures to exhibit their species-specific behavior. A few studies investigated MO effect on the weaned rabbits' performance and behavior (Elkloub *et al*., 2018; Maken *et al*., 2022; Maher *et al*., 2023), hence the current objective of the research intended for evaluating the role of MO in the reduction of weaning stress via evaluating its effect on behavior, performance, oxidative statue, and liver and kidney function.

Materials and methods

Moringa Oleifera leaves (MO) preparation and incorporation in rabbit feed

Fresh moringa leaves were collected from the moringa trees at the Land of Beni-Suef University's Faculty of Agriculture. Then, the leaves were rinsed with water and left to dry under solar rays. The dried leaves are used to substitute a part of rabbit feed protein and fiber source (soybean and wheat straw) in a 5% percent during pellet manufacturing by Omara *et al*. (2018).

Animals and Experimental Design

Twelve recently weaned V-Line rabbit bunnies about 4 weeks of age and average body weight of 0.74±0.07 were raised in batteries in a private rabbit facility. The mean house temperature was 23±2°C and the approximate relative humidity was 45-55%.

The recently weaned bunnies were separated equally to feed either normal-prepared rabbit feed (Control group (C); n=6) or moringa-prepared feed (*Moringa Oleifera* group (MO); n=6) for 4 weeks, throughout the experimental, either moringa-prepared feed or normal feed along with clean drinking water (by using nipples) were offered, and the bunnies were maintained under sanitary situations. This work was granted approval by the Beni-Suef University College of Veterinary Medicine ethical review board with Approval number (022-452).

Growth performance assessment

Feed intake

Daily feed intake was recorded and then, weekly feed intake (FI per kg) was calculated using a simple formula:
Feed Intake/Kg= Feed introduced/Kg – Residual feed/Kg, as stated by Selim *et al*. (2021).

Body weight and weight gain

Every week, we weighed each bunny individually using a digital scale in the morning before they had food or water. We did this at a fixed time to reduce variations and get accurate weight measurements (known as "fasted weight"). To calculate the body weight gain (BWG, in kilograms) of each bunny, we simply subtracted its current weight from its previous weight.

Behavioral patterns recording and analysis

To mark the bunnies, their foreheads were colored with special permanent markers. A blind observer recorded their behavior using a digital video camera, while standing about a meter away from the rabbits' battery, to avoid disturbing the animals (Bateson and Martin, 2021). Each group was videotaped in the morning and evening for continuous thirty minutes during feeding, three days a week. Afterward, different behavioral patterns were manually analyzed by focusing on individual rabbits (Gunn and Morton, 1995; Prebble *et al*., 2015), as shown in Table 3.

Blood sampling and serum biochemical analysis

A sample of blood was drawn from each rabbit's ear veins into tubes without anticoagulant. The serum then obtained by centrifugation and stored at -20°C in a labeled clean Eppendorf tube for biochemical analysis.

Total protein was measured colorimetrically using the Biuret reaction described by Henry (1964). Albumin was measured using a photometric colorimetric method in accordance with Doumas *et al*. (1971).

To determine the cholesterol level, the enzymatic analysis method developed by Röschlau *et al*. in 1974 was utilized., as described by Schumann and Klauke (2003) kinetic method, was used to assess ALT (alanine aminotransferase) and AST (aspartate aminotransferase) activities. The measurement of creatinine levels followed the protocols established by Mazzachi *et al*. (2000) and Rifai (2017).

The estimation of serum lipid peroxidation was carried out by colorimetric measurement for the content of malondialdehyde (MDA) in serum, following the method of Albro *et al*. (1986). Additionally, the content of reduced glutathione (GSH) was determined by the method of Ellman (1959).

Statistical analysis

The data was analyzed using T-tests using SPSS v22. The harvested data were presented as mean±SE and considered significant at $P < 0.05$.

Results

By noticing the influence of MO on the behavior of weaned bunnies, it was clear that feeding frequency, feeding duration, drinking frequency, drinking duration and frequency of activity were elevated significantly in MO group compared to C group at (P=0.000; P=0.003; P=0.001; P=0.000; P=0.03) in order while resting duration was diminished in MO group than C group at (P=0.000) as showed in Figure 1(A, B).

Table 1. The ingredients of the normal rabbit feed

Feed composition (%)	Soybean 44%	Wheat bran	Yellow corn	Wheat straw	Clover hay	Barley	Premix	Total
	18.5	32	14	9	20	5	1.5	100
Chemical composition (%)	Crude protein	Crude fiber	Ash	Moisture	Metabolizable energy (Kcal/kg)			
	17.1	13.5	6.7	6.8	2520			

Table 2. The ingredients of the moringa-prepared rabbit feed

Feed composition (%)	Soybean 44%	Wheat bran	Yellow corn	Wheat straw	Moringa	Clover hay	Barley	Premix	Total
	16	32	14	7	5	20	5	1	100
Chemical composition (%)	Crude protein	Crude fiber	Ash	Moisture	Metabolizable energy (Kcal/kg)				
	17.1	13.7	6.5	6.2	2490				

From Figure 2 (A), data showed that the statistical difference was absent between the MO and C groups in the body weight, however in the second week, MO group showed enhanced body weight compared to C group (P=0.03). Additionally, no significant difference was observed between the two groups either in feed intake of the amount of consumed rabbit ration or in weight gain of rabbits Figure 2 (B).

The noticed results in Table 4 showed that MO feed didn't alter the lipid peroxidation (MDA) and antioxidant activities (GSH) of treated rabbits when compared to the C group.

By assessment of hepatic and renal function, data demonstrated that MO supplementation induced a marked elevation in the serum albumin than C group (P=0.02). In addition, the MO feed induced a marked reduction in cholesterol level and ALT level than the C group (P=0.000; P=0.002) respectively as obvious in Table 5.

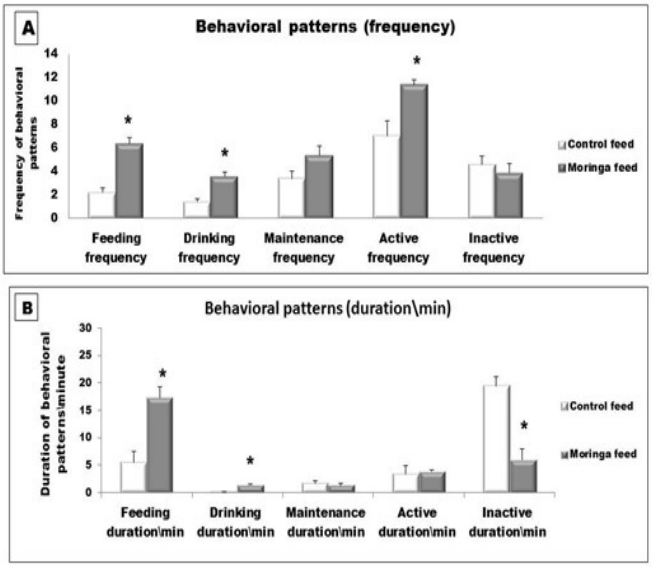

Figure 1. Influence of MO on different behaviors of the weaned rabbits; (A) Behavioral patterns frequency, (B) Behavioral patterns duration\minute.
Results are expressed as Mean±SE, and a stride indicates significance in different behavioral patterns between the control and moringa groups at ($P < 0.01$), while Active frequency was significant at ($P < 0.05$).

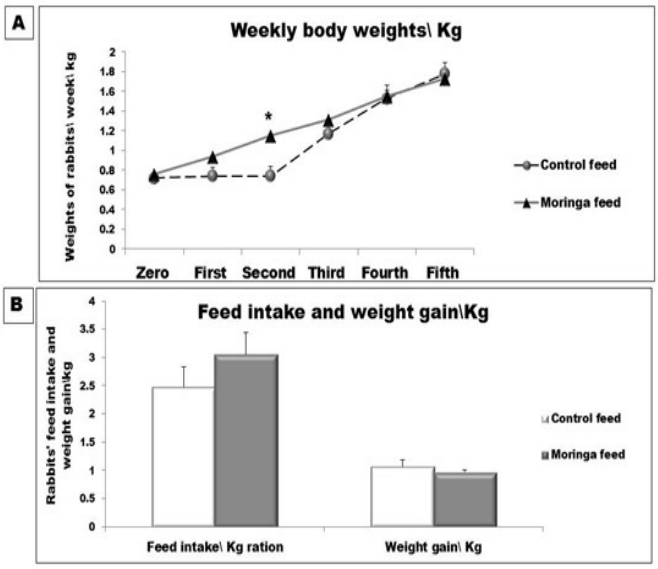

Figure 2. Influence of MO on the performance of the weaned rabbits; (A) Weekly body weight\ kg, (B) Feed intake and weight gain\ kg.
Results are expressed as Mean±SE, and a stride indicates significance between the control and moringa groups at ($P < 0.05$).

Table 3. Behavioral Ethogram

Behavioral Patterns	Behavior Description
Feeding	Eating or chewing pellets from the feeder
Drinking	Nosing or drinking from water nipple
Maintenance	Grooming and Licking of the body
Active	Hopping, Chasing, Jumping
Inactive	Lying, Sitting

Table 4. Influence of MO on the oxidative stress in rabbits' serum.

Oxidative parameters	Control	Moringa
MDA (nmol/ml)	0.30±0.03	0.39±0.03
GSH (Mmol\ml)	0.04±0.00	0.03±0.01

Results are expressed as Mean±SE

Table 5. Influence of MO on the hepatic and renal function in rabbits' serum

Liver and kidney function	Control	Moringa
Total protein (g/dl)	5.08±0.27	5.03±0.27
Albumin (g/dl)	2.83±0.20	4.27±0.30*
ALT (U/L)	31.78±1.13*	19.33±1.20
AST (U/L)	19.44±0.80	18.67±0.88
Cholesterol (mg/dl)	216.00±1.73*	103.83±2.20
Creatinine (mg/dl)	0.56±0.09	0.46±0.07

Results were expressed as Mean±SE, and a stride indicates significance in between the control and moringa groups at ($P < 0.01$), while the albumin level was significant at ($P < 0.05$).
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

Discussion

Evaluating animal behavior, a key indicator of welfare is crucial to assess deviations from species-specific behavior (Jordan *et al*., 2011). According to El-Tazi (2014) and Ramadan (2017), feeding behavior in rabbits increased significantly when fed with a MO diet containing 5% of the ingredients. Additionally, Lashari *et al.* (2021) found that low doses of MO encouraged normal rabbit behaviors and increased feeding behavior, whereas high doses did not have the same effect.

In the MO group, the frequency and duration of drinking behavior increased significantly when compared to the C group. This may be due to the significant elevation in albumin levels of the growing rabbits treated with MO (Abdella and Khalifah, 2021; El-Kashef, 2022). El-Desoky *et al.* (2017) mentioned that blood protein albumin has a small molecular weight and a significant residual negative charge at pH because of its chemical makeup, it can be an extremely hydrophilic molecule that accounts for between 75 and 80 percentage of the vascular colloidal osmotic pressure and plays a crucial role in maintaining tissue fluid homeostasis., Moreover, Lashari *et al.* (2021) reported that a low MO diet resulted in high grooming behavior, decreased lie recumbent time, and high non-significant locomotion. Furthermore, Zade *et al.* (2013) found that various MO extracts decreased non-genital grooming in treated MO male rats.

In the current study, MO feed group showed a non-significant increase in the feed intake than the control group that run in parallel with Safwat *et al.* (2014) recorded that high feed was observed in MO group and control with the absence of statistical difference, and Awodele *et al.* (2012) revealed a non-significant difference in the weight gain between C and MO treated animals. Similarly, the feed intake, and weight gain didn't differ significantly by supplementation of MO as previously reported by Bakr *et al.* (2019). El-Desoky *et al.* (2018) and Mankga *et al.* (2022) who observed an enhancement in the body weight of rabbits by incorporating MO into the rabbit diet.

The blood biochemical profiles are considered as a reliable means to evaluate the well-being of animals and additionally indicate the biological responses of the animal towards its both internal and external circumstances, particularly dietary intake and feeding (Church *et al*., 1984; Bakr *et al*., 2019). Abd-Elnaby *et al.* (2022) found that MO treatment didn't alter the MDA and GSH levels compared to control rats. Concerning the effect of MO on renal function, Selim *et al.* (2021) demonstrated that MO didn't alter the creatinine serum level as compared to the control group.

The total protein and albumin levels elevation illustrates this plant's potential to boost the breakdown of proteins and accelerate the regenerative process of the liver tissue (El-Kashef, 2022), our results showed an

elevation in albumin serum levels of MO group as previously reported by Omara *et al*. (2018) and El-Kashef (2022). Moreover, the supplementation of MO resulted in a significant reduction in serum cholesterol due to its hypocholesterolemic effect, and this agree with Jain *et al*. (2010); Samar *et al*. (2016); Adeyemi (2018); Omara *et al*. (2018) and El-Kashef (2022).

In certain instances of chronic liver illness, the activitiy of ALT was higher than the activity of AST (Anadón *et al*., 2019). Mildly elevated ALT activity in sound rabbits has been associated with frequent exposure to low doses of toxins, like resins in wood litter or aflatoxins in the diet (Fekete and Huszenicza, 1993). In the current study it was clear that MO decreased serum ALT activity in rabbits which runs in agreement with El-Desoky *et al*. (2021) and El-Kashef (2022) and explains moringa's role in preserving the health and safety of liver tissues. Moreover, Varga, 2014 stated that weaning stress may cause digestive troubles, the levels of ammonia in the caecum reduced after weaning because of the change feeding from milk to solids is attributed to the acidic pH of the caecum as the volatile fatty acid levels rise (Varga, 2014; Kazana *et al*., 2022). The increased volatile fatty acid levels and elevated ALT serum activity may be indications for liver damage or fatty liver that is accompanied by enzyme disturbance (high ALT) and metabolic disturbance (high cholesterol) because of weaning stress. MO has antioxidant, nephroprotective, and hepatoprotective effects that help in the alleviation of weaning stress negative impacts.

Conclusion

The incorporation of *Moringa Oleifera* leaves in the recently weaned rabbits' diet as an alternative to antibiotics has a beneficial role in reducing the negative consequences of antibiotics residue on rabbit and human health, in addition to their role in the weaning stress alleviation via their antioxidant, hepatic and renal function preservation, and their positive effects on rabbit bunnies' performance and behavior.

Conflict of interest

The authors declare that they have no conflict of interest.

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