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Dietary Supplementation of *Moringa Oleifera* Leaves and Their Nanoparticles to Rabbit Does Altered the Neonates Performance, Behavioural and Physiological Response to Stress

Mahmoud Maher[1], Asmaa K. Abdelghany[1*], Masouda A. Allak[2], H.H. Emeash[1], Fatma Khalil[1]
[1]Animal and Poultry Management and Wealth Development Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, 62511, Egypt
[2]Animal Production Department, Faculty of Agriculture, Fayoum University, Egypt
*Corresponding Author: Asmaa K. Abdelghany, E-Mail: asmaa.kamal@vet.bsu.edu.eg

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ABSTRACT

This study was conducted to investigate the effects of supplementing rabbits does with nano-zinc (NZn), nano-moringa (NMo), or moringa extract (MoE) on their growing rabbits (GR; fed on a moringa diet) behaviour, performance, and weaning stress alleviation. Twenty four does were equally divided into 4 groups; NZn-treated dams, NMo-treated dams, moringa extract MoE-treated dams, and non-treated dams (control; NTD) throughout gestation, lactation, and until the weaning of neonates. Immediately after weaning at about 35 days of age, 48 GR [12 from each treated dam group] were subdivided into 8 equal groups (each 12 GR/gp divided into 2 sub-groups: n = 6 per each); one sub group fed on a basal diet (BD) and the other fed on a 5% *Moringa olifera*-containing ration (MD) for 4 weeks. Feed intake (FI), body weight, and behavioural changes in the GR were recorded. Blood samples were collected at the termination of the experiment to assess oxidative stress (MDA, GSH), growth indicators (T3, T4), and liver and kidney functions. As a result, MD supplementation significantly (P < 0.01) increased FI compared to BD in the GR from control and NZn-treated dams. In addition, MD supplementation resulted in increased feeding and drinking behaviours frequency and duration, while self-grooming was reduced in both NTD and NZn-treated dams in relation to BD supplemented groups. Moreover, MD significantly decreased oxidative stress in GR from NTD. Also, MD induced a significant (P < 0.01) increase in T3 level and enhanced the liver and kidney functions in GR from all treated dams compared to control. Furthermore, MD supplementation increased the economic efficiency of GR. In conclusion, MD supplementation to GRs or treatment of their dams with the mentioned treatments decreased weaning stress, improved rabbit behaviour, and improved economic efficiency, but no significant improvement in the GRs' performance was observed. However, treatments of dams and their GR with MD resulted in GR inability to cope with weaning stress and induced renal and hepatic damage. Thus, it is recommended to supplement GR with MD after weaning or prenatal treatment of their dams with herbal extract or nano-herbal particles, but a combination of both treatments is not recommended.

Keywords: *Moringa Oleifera*, Nano-Zinc, Performance, Rabbit.

INTRODUCTION

Globally, there is an increase in the demand for cheap and high quality animal protein. The lack of animal protein sources, particularly in developing nations, may be linked to an increase in livestock production costs **(Etchu *et al*., 2017)**.

The rabbit (*Oryctolagus cuniculus*) produces the highest-quality lean meat, which provides necessary amino acids, polyunsaturated fatty acids, vitamins, and minerals, is lower in cholesterol, and lacks uric acid (**Etchu *et al*., 2017; Selim *et al*., 202; El-Ratel *et al*., 2023**).

Growth promoters are commonly used to enhance feed conversion, performance, and meat quality **(Islam *et al*., 2022)**. The European restriction of growth promoters containing antibiotics and their harmful effects motivated researchers and feed organizations to investigate novel animal feeding methods **(Dalle Zotte *et al*., 2016)**. Phytochemical components, phytogenic feed additives and/or extracts are used in animal nutrition to improve digestive enzyme output, activate the immune system, and promote antibacterial and antioxidant qualities **(Hashem *et al*., 2013; Dalle Zotte *et al*., 2016; Abou-Elkhair *et al*., 2018)**.

Feed additives are used to enhance meat production **(Islam *et al*., 2022).** Among the used feed additives is *Moringa oleifera* which is grown extensively in Africa, Asia, and the United States; it is also commonly grown in Egypt due to its adaptability to a variety of environmental conditions and soil types **(Saalu *et al*., 2011; Selim *et al*., 2021)**. *Moringa oleifera* leaves (MOL) have a high concentration of all necessary and sulfur-rich amino acids, and true protein comprises about 87% of the total protein content **(El-Desoky *et al*., 2018)** and is rich in minerals , vitamins, and natural antioxidants **(Gowrishankar *et al*., 2010)**.

Nowadays, nanotechnology is more frequently used in all different fields, including animal production. Many studies indicated that using nanoparticles has a beneficial effect on animal production and performance, Zinc and its nanoparticles (NPs) were used as antioxidants, anti-inflammatory agents, immunoregulators, and to improve the performance of animals **(Hassan *et al*., 2017)**. Nano-sizing of metals or their use as herbal nano-carriers has a potential to enhance their activity, overcome problems associated with plant absorption, and direct them to the target site of action **(Ansari, *et al*., 2012; Bonifacio *et al*., 2014)**. However, exposure to zinc and zinc oxide nanoparticles may increase the risk of their toxicity **(Igarashi, 2008)**. For example, nephrotoxicity and changes in renal function resulted from high levels of zinc oxide nanoparticles "ZnO NPs" in laboratory animals **(Sharma *et al*., 2012)**. Moringa Oleifera leaves' extract acted as both reducing and stabilizing agents during the synthesis of nanocomposites of Zn2+ via its bioactive compounds **(Ngom *et al*., 2021)**.

Behaviour of individuals, such as feeding and self-grooming, reflects animals dietary intake **(Lashari *et al*., 2021**), anxiety, and stress in rodents **(Song *et al*., 2016).** Early studies pointed to the improvement effect of MOL on the average bunny weight **(Emmanuel *et al*., 2014)**. In addition, **Omara *et al*. (2018)** showed that MOL altered the blood biochemical parameters in rabbits. However, so far, there have been no studies comparing the effects of MOL and their nanoparticles on the behaviour and performance of rabbits.

Therefore, the current study was designated to compare the effect of supplementing dams (during pregnancy and lactation) with MOL extract, Nano-moringa, and Nano-zinc particles on alleviating weaning stress in their neonates with and without feeding them on MOL containing diets for 4 weeks. Performance, behaviour, and physiological response to stress were evaluated via antioxidant activity, thyroid hormones, and kidney and liver functions in growing rabbits.

MATERIALS AND METHODS

1. Moringa diet and nanoparticle preparation

Fresh MOL was harvested at morning from agro-forestry at agriculture factory- Beni-suef University. Later, leaves were washed by tap water followed by distilled water and allowed to dry by solar rays. The dried MOL was included as a component in the rabbits ration formulation during pellets manufacturing by a percent of 5% according to **Omara *et al*. (2018)**. Aqueous extract of *Moringa Olifera* was prepared, Nano-Zinc and Nano-Moringa leaves particles were prepared in the Nanotechnology Department in the Faculty of Postgraduate, Beni-Suef University, Egypt.

2. Animals

Twenty four nulliparous V-Line rabbit does about 4.6 months of age with average body weight 3.10 ± 0.20 were reared in a commercial rabbit farm exposed to one of the following treatments; Nano zinc, Nano moringa, moringa extract or distilled water via oral gavage during gestation period and till weaning of neonates at age of one month. After weaning of neonates, forty eight male and female growing rabbits (GR) (12 from each group; with initial average live weight; 0.75-0.78kg\ neonates were used for the current study). The average temperature was 20 ± 1.5^{0}C and relative humidity (RH) 49±3%. Provision of prepared rabbit ration and clean fresh water (through nipples) were maintained throughout the experiment. The rabbits were kept under hygienic environmental conditions.

3. Ethical approval

The ethical committee of the college of veterinary medicine at Beni-Suef University provided approval for this work under Approval number (022-316).

4. Experimental design

The rabbit does were orally gavaged at a fixed time (8:00 am) with Nano-Zinc (NZn), Nano-Moringa (NMo), or Moringa Extract (MoE) during pregnancy and lactation, while their weaned neonates were fed on the MD or BD diet for 4 weeks, followed by assessment of their behaviour and performance in a trial to alleviate the weaning stress using MOL, as described in Fig. 1.

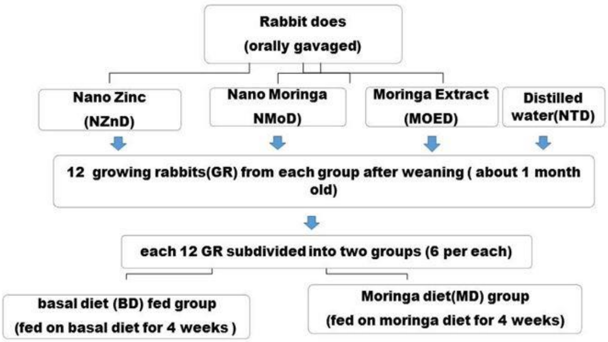

Fig 1: Scheme of the experimental design

5. Diet composition

Table 1: Ingredients of experimental diets (%)

Diet composition	Basal diet	Moringa diet
Soybean meal 44%	18.5	16
Wheat bran	32	32
Yellow corn grain	14	14
Wheat straw	9	7
Clover hay	20	20
Barley	5	5
Moringa leaves	0	5
Premix	1.5	1
Total	100	100
Chemical composition (%) according to National Research Council (NRC) 1977		
Crude protein	17.1	17.1
Metabolizable Energy "ME" (Kcal/kg)	2520	2490
Crude fiber	13.5	13.7
Ash	6.7	6.5
Moisture	6.8	6.2

Each one kg of premix (minerals and vitamins mixture) contains vitamin A, 20000 IU; vitamin D3, 15000 IU; vitamin E, 8.33 g; vitamin K, 0.33 g; vitamin B1, 0.33; vit. B2, 1.0 g; vitamin B6, 0.33 g; vitamin B5, 8.33 g; vitamin B12, 1.7 mg; pantothenic acid, 3.33 g; biotine, 33 mg; folic acid, 0.83 g; choline chloride, 200 mg; Growel Agrovet, Egypt.

6. Identification of cages and growing rabbits

Two ways of identification was used, cage identification using cage card and animal identification by painting them with special pigments such as saturated carbol fuchsin, Giemsa, and malachite green stains on the

7. Growth performance parameters

7.1. Feed intake and Feed Conversion ratio

Daily feed intake (FI, kg) was calculated weekly **(Selim *et al*., 2021):**

Feed Intake/Kg = Feed introduced/Kg– Residual feed/Kg

Feed Conversion ratio (FCR, kg/kg gain) was calculated through this equation:

$$F.C.R = \frac{\text{Feed intake}}{\text{Final weight} - \text{initial weight}}$$

7.2. Body weight

Rabbits were weighed individually using a digital scale in the morning before feeding or drinking at fixed time of day to reduce variations and obtain accurate weight (Fasted weight) once every week. Then, the body weight gain (BWG, kg) was calculated by subtracting the current weight from the previous weight.

8. Behaviour assessment

The rabbit behaviour was recorded by blind observer using a digital video camera and standing about 1 meter distance from the rabbits' battery to avoid the incidence of disturbance to animals **(Martin *et al*., 1993)**. Each group was videotaped for thirty minutes during feeding twice daily, three days weekly. Then, different behavioural patterns were analyzed manually by focal observation of individual rabbits as mentioned by **Dal Bosco *et al*., (2019)** in **Table 2**.

Table 2: Behavioural Ethogram (by recording frequency and duration/minute).

Behaviour Patterns	Behaviour Description
Eating	Head above the feeder. Eating or chewing pellets
Drinking	Head in close proximity to water nipple. Nosing or drinking from water nipple
Self-grooming	Licking, scratching, or nibbling of the body

9. Blood sampling

At the end of the experiment, blood samples were obtained from the rabbits' ear vein inside tubes lacking anticoagulant, centrifuged to extract serum, and then preserved at -20 °C in a markedly clean E pindoorf for physiological and biochemical calculations.

9.1. Determination of blood biochemical and metabolite parameters

Total protein **(Henry, 1964)**, albumin (**Doumas *et al*., 1971),** globulin (subtraction of albumin from total protein), cholesterol (**Röschlau *et al*., 1974)**, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) **(Schumann and Klauke., 2003),** and creatinine (**Mazzachi *et al.* 2000; Rifai, 2017)** were determined to evaluate any changes in their levels**.** Chemiluminescence immunoassay (ECLIA) technology was followed for quantitative assessments of triiodothyronine (T3), thyroxine (T4).

9.2. Determination of antioxidant activity

Lipid peroxidation in the serum was estimated by the colorimetric method by measuring serum malondialdehyde (MDA) content, as described by **Albro *et al.* (1986)**. Also, reduced hepatic glutathione (GSH) content was measured according to **Ellman (1959)**.

10.Calculation of Economic Efficiency

Economic Efficiency (E.E) for meat production was calculated as described by **(El-Desoky *et al.*, 2018).**

11. Statistical analysis

All data were analyzed by one way analysis of variance (One way ANOVA) using SPSS version 22 statistical software (IBM Corp. Released 2011. IBM SPSS statistics for window, version 22.0 Armonk, NY: IBM Corp). The obtained data were presented as mean±SE and considered significant at $P<0.05$.

RESULTS

Table (3) showed that at the fourth week post feeding of GR (from NTD) on MD, FI was significantly ($P<0.01$) increased when compared to BD in the same group. However, MD significantly ($P < 0.01, 0.05$) decreased the FI of GR from NMoD and MoED, respectively, compared to the BD-GR of each group. In addition, the FI of GR (from NZnD, NMoD, and MoED) fed on BD was significantly ($P <0.01$) increased in comparison with those fed BD in NTD.

Table 3: Effects of *Moringa oleifera* leaves on feed intake (Kg) and feed conversion rate (Kg/Kg gain) of growing rabbits delivered from dams treated by Nanozinc, Nano-Moringa and *Moringa oleifera* leaves extract during pregnancy and lactation.

Period of feeding growing rabbit / week		Treatment of dams								Significance					
		Control (NTD)		NZnD		NMoD		MoED		Treatments *VS* Control (NTD)					
		Growing rabbits diets								NZnD		NMoD		MoED	
		BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD
Feed intake (Kg)	First	0.29 ± 0.03	0.29 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	NS	NS	NS	NS	NS	NS
	Second	0.33 ± 0.03	0.36 ± 0.03	0.32 ± 0.03	0.35 ± 0.04	0.33 ± 0.03	0.36 ± 0.03	0.34 ± 0.03	0.35 ± 0.03	NS	NS	NS	NS	NS	NS
	Third	0.41 ± 0.04	0.45 ± 0.03	0.40 ± 0.03	0.46 ± 0.03	0.44 ± 0.03	0.43 ± 0.03	0.47 ± 0.03	0.45 ± 0.03	NS	NS	NS	NS	NS	NS
	Fourth	0.40 ± 0.03	$0.57 \pm 0.01^{**}$	0.60 ± 0.01	0.57 ± 0.01	0.57 ± 0.02	$0.45 \pm 0.01^{**}$	0.59 ± 0.01	$0.54 \pm 0.01^{*}$	**	NS	**	**	**	NS
Feed conversion ratio (Kg/Kg gain)		4.45 ± 1.81	2.46 ± 0.03	2.68 ± 0.19	2.45 ± 0.12	2.58 ± 0.27	2.43 ± 0.27	2.67 ± 0.04	1.91 ± 0.38	NS	NS	NS	NS	NS	NS

* = *P* value <0.05   ** = *P value <0.01*
NTD = Non Treated Dams   NZnD = Nano-Zinc treated Dams   NMoD = Nano-Moringa treated Dams
MoED = Moringa Extract treated Dams
BD = Basal Diet   MD = Moringa containing Diet   NS = Non significant   FCR = Feed Conversion ratio.

Table (4) declared that there was no significant difference in weight and weight gain in GR from all groups throughout the period of feeding on MD.

Table 4: Effects of *Moringa oleifera* leaves on Body weight (Kg) and weight gain (Kg) of growing rabbits delivered from dams treated by Nanozinc, Nano-Moringa and *Moringa oleifera* leaves extract during gestation and lactation period.

Period of feeding growing rabbit / week	Treatment of dams								Significance					
	Control (NTD)		NZnD		NMoD		MoED		Treatments *VS* Control (NTD)					
	Growing rabbits diets								NZnD		NMoD		MoED	
	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD
Zero	0.75 ± 0.03	0.75 ± 0.03	0.76 ± 0.01	0.76 ± 0.00	0.77 ± 0.02	0.77 ± 0.02	0.75 ± 0.01	0.75 ± 0.06	NS	NS	NS	NS	NS	NS
First	0.93 ± 0.04	0.97 ± 0.01	0.93 ± 0.01	0.95 ± 0.01	0.93 ± 0.03	0.94 ± 0.02	0.94 ± 0.00	0.93 ± 0.07	NS	NS	NS	NS	NS	NS
Second	1.11 ± 0.06	1.14 ± 0.01	1.09 ± 0.05	1.10 ± 0.02	1.17 ± 0.04	1.19 ± 0.02	1.09 ± 0.01	1.18 ± 0.05	NS	NS	NS	NS	NS	NS
Third	1.33 ± 0.10	1.33 ± 0.01	1.28 ± 0.04	1.35 ± 0.02	1.36 ± 0.04	1.38 ± 0.03	1.26 ± 0.02	1.37 ± 0.05	NS	NS	NS	NS	NS	NS
Fourth	1.42 ± 0.16	1.58 ± 0.01	1.61 ± 0.05	1.60 ± 0.03	1.63 ± 0.05	1.60 ± 0.06	1.53 ± 0.02	1.64 ± 0.07	NS	NS	NS	NS	NS	NS
Total weight gain (Kg)	0.68 ± 0.15	0.83 ± 0.02	0.85 ± 0.05	0.85 ± 0.03	0.86 ± 0.03	0.82 ± 0.06	0.78 ± 0.01	0.88 ± 0.03	NS	NS	NS	NS	NS	NS

NTD = Non Treated Dams NZnD = Nano-Zinc treated Dams NMoD = Nano-Moringa treated Dams
MoED = Moringa Extract treated Dams
BD = Basal Diet MD = Moringa containing Diet NS = Non significant * = *P* value <0.05
** = *P* value <0.01.

Results in table **(5)** declare the statistical alterations in behaviours of GR harvested from treated dams with nanoparticles and MOL extract. It was obvious that GR from NTD and NZnD, which fed on MD, exhibited a significant (*P <0.01*) increase in feeding duration compared to BD of the same group. GR from NZnD, NMoD, and MoED fed with BD spent a significant (*P <0.01*) longer time feeding than those in BD of the NTD group. However, the time of feeding of the NZnD, NMoD, and MoED groups was significantly (*P <0.01*) shorter than the MD of the NTD group. Drinking behaviour of GR was markedly affected by different treatments of dams, as drinking frequency was significantly (*P <0.05, 0.01*) increased with MD in GR from NTD and MoED compared to that of the fed on BD of the same group. MD in both NTD and NZnD induced a significant (*P <0.05, 0.01*) increase in the time of drinking in GR as compared to BD in the same group. Similarly, drinking time significantly (*P <0.05*) increased in NZnD in relation to the BD of the NTD.

In addition, grooming frequency and duration were significantly (*P* =0.03, 0.00) diminished in GR from NTD fed on MD compared to BD of the same group. However, they significantly (*P <0.01*) increased in GR from MoED and fed on MD in relation to BD in the same group. Grooming frequency was significantly (*P <0.05, 0.01*) increased in GR from both NMoD and MoED fed on MD compared to MD from NTD. In addition, they significantly (*P <0.01*) decreased in GR from MoED and fed on BD compared to BD of NTD. The grooming duration decreased significantly (*P <0.01, 0.05, 0.01*) in GR fed on BD from NZnD, NMoD, and MoED as compared to those in NTD in order. However, MD significantly (*P <0.01*) increased grooming duration in MoED compared to MD in NTD.

Table 5: Effects of *Moringa oleifera* leaves on behaviours of growing rabbits delivered from dams treated by Nanozinc, Nano-Moringa and *Moringa oleifera* leaves extract during gestation and lactation period.

Behavioural Patterns			Treatment of dams								Significance					
			Control (NTD)		NZnD		NMoD		MoED		Treatment *vs.* control					
			Growing rabbits diets								NZnD		NMoD		MoED	
			BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD
Ingestive	Feeding	Frequency	3.60 ± 2.46	5.00 ± 1.67	4.80 ± 1.20	5.17 ± 0.75	4.90 ± 0.99	5.83 ± 1.60	3.88 ± 0.49	4.67 ± 1.63	NS	NS	NS	NS	NS	NS
Ingestive	Feeding	Duration \minute	10.69 ± 1.96	22.67 ± 1.34**	15.20 ± 1.90	19.56 ± 2.63**	16.79 ± 2.80	17.65 ± 3.21	16.19 ± 1.96	15.58 ± 2.05	**	*	**	**	**	**
Ingestive	Drinking	Frequency	1.20 ± 1.04	2.30 ± 1.12*	1.40 ± 1.19	2.13 ± 0.43	1.22 ± 0.51	2.03 ± 0.30	1.33 ± 0.62	2.80 ± 1.10**	NS	NS	NS	NS	NS	NS
Ingestive	Drinking	Duration \minute	0.33 ± 0.26	1.45 ± 0.43**	1.23 ± 0.88	1.99 ± 0.74*	0.72 ± 0.56	1.34 ± 0.86	1.06 ± 0.50	0.90 ± 0.45	*	NS	NS	NS	NS	NS
Self-grooming		Frequency	4.33 ± 2.50	2.47 ± 0.85*	3.65 ± 1.67	4.00 ± 1.31	2.77 ± 1.18	4.16 ± 0.69	2.33 ± 0.85	5.23 ± 1.34**	NS	NS	NS	*	*	**
Self-grooming		Duration \minute	2.26 ± 1.23	0.44 ± 0.20**	0.86 ± 0.50	0.77 ± 0.39	1.40 ± 1.10	0.75 ± 0.32	0.63 ± 0.24	1.87 ± 0.34**	**	NS	*	NS	**	**

Data expressed as mean± SE
NTD = Non Treated Dams NZnD = Nano-Zinc treated Dams NMoD = Nano-Moringa treated Dams MoED = Moringa Extract treated Dams
BD = Basal Diet MD = Moringa containing Diet NS = Non significant
*= *P* value <0.05 ** = *P* value <0.01.

Table **(**6) declares the statistical changes in antioxidant activity and thyroid hormone levels of GR in response to prenatal and postnatal treatments. Regarding antioxidant activity, MD significantly (*P < 0.01*) decreased the MDA level in GR from NTD in relation to BD in the same group. In addition, MDA level was significantly (*P < 0.01*) decreased in GR from NZnD fed on BD and MD compared to BD from NTD. Moreover, MDA levels were reduced significantly (*P < 0.01*) in GR form of NMoD fed on BD.

Furthermore, MD significantly (*P < 0.01*) increased the level of GSH in GR from all groups (NTD,NZnD, NMoD, and MoED) when compared to BD in the same groups. However, by comparing them with the control group, BD and MD significantly (*P <0.01*) decreased the GSH level in GR from NZnD, and BD and MD significantly (*P <0.01*) decreased the level of GSH in GR from MoED, respectively. Concerning thyroid hormone level alterations, MD significantly (*P <0.01*) increased T3 level in GR from MoED, and MD significantly (*P <0.05*) increased T3 level in GR from NZnD and MoEd, respectively, compared to the control group.

Table 6: Effects of *Moringa oleifera* leaves on antioxidant activity and thyroid hormones of growing rabbits delivered from dams treated by Nanozinc, Nano-Moringa and *Moringa oleifera* leaves during gestation and lactation period.

Parameters		Treatment of dams								Significance					
		Control (NTD)		NZnD		NMoD		MoED		Treatment *vs.* control					
		Growing rabbits diets								NZnD		NMoD		MoED	
		BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD
antioxidant activity	MDA (nmol/ml)	0.47 ± 0.03	0.37 ± 0.05*	0.22 ± 0.04	0.23 ± 0.04	0.26 ± 0.01	0.29 ± 0.01	0.42 ± 0.02	0.35 ± 0.02	**	**	**	NS	NS	NS
	GSH (nmol/ml)	26.48 ± 2.67	40.86 ± 2.12**	10.76 ± 0.34	24.55 ± 1.84**	26.66 ± 1.92	40.49 ± 1.24**	21.10 ± 1.24	30.07 ± 0.58**	**	**	NS	NS	*	**
T3 (µg/dl)		35.63 ± 3.26	33.57 ± 1.65	37.63 ± 2.46	42.13 ± 1.52	35.03 ± 1.92	32.27 ± 1.31	37.80 ± 2.48	51.90 ± 2.31**	NS	*	NS	NS	NS	**
T4 (ng/dl)		2.40 ± 0.23	2.63 ± 0.39	2.73 ± 0.35	2.73 ± 0.18	2.37 ± 0.13	2.57 ± 0.52	2.40 ± 0.31	3.07 ± 0.47	NS	NS	NS	NS	NS	NS

Data expressed as mean± **SE**
NTD = Non Treated Dams NZnD = Nano-Zinc treated Dams NMoD = Nano-Moringa treated Dams
MoED = Moringa Extract treated Dams BD = Basal Diet MD = Moringa containing Diet
NS = Non Significance *= *P* value <0.05 ** = *P* value <0.01
MDA = Malondialdehyde GSH = Reduced glutathione T3 = Triiodothyronine T4= Thyroxine.

Table (7) showed that MD significantly (*P <0.05*) increased the protein level of GR from NTD in relation to BD in the same group. However, GR from other treated dams showed no significant alteration in total protein levels. MD significantly *(P < 0.01*) increased the albumin level of GR in all groups compared to BD in each group. Additionally, MD-GR from NZnD significantly (*P <0.05*) increased albumin levels in comparison to the control group. The globulin level was significantly (*P <0.01*) elevated in MD-GR from NTD in relation to BD-GR in the same group. In spite of MD, there was a significantly (*P < 0.05*) lower globulin level in GR from NZnD and MoED in comparison with the control group.

MD significantly (*P <0.01*) decreased the cholesterol level of GR in all groups when compared to BD in these groups. However, BD significantly (*P < 0.01*) lowers the cholesterol level of GR from NZnD, NMoD, and MoED in comparison to the control group, while MD significantly (*P < 0.01*, *0.05*) decreases the cholesterol level of GR from NMoD and MoED, respectively, and increases (*P < 0.01*) its level in GR from NZnD in comparison to the control group.

Liver enzymes activity in GR showed statistical differences between treatments. MD significantly (*P < 0.05, 0.01*) decreased ALT and AST activity in GR from NTD and NMoD, respectively, when compared to BD in the same groups. However, MD induced a significant (*P < 0.05, 0.01*) increase in the activity of ALT and AST in GR from NZnD. In addition, AST in GR from MoED fed on MD showed a significant (*P < 0.01*) increase in relation to BD in the same groups. ALT in BD-GR from NZnD and NMoD was significantly (*P < 0.01*) increased compared to BD of NTD. Also, AST was significantly (*P < 0.01*) increased and decreased significantly in GR from NMoD and MoED, respectively. While ALT and AST activity were significantly (*P < 0.01*) increased in MD-GR from NZnD and MoED and (*P < 0.05*) in MD-GR from NMoD in relation to the control group.

Regarding kidney function, creatinine levels were significantly (*P < 0.01*) high in GR harvested from NZnD and MoED, and fed on BD and MD, respectively. GR from NZnD and NMoD fed on BD showed a significant (*P <0.01*) elevation in the creatinine level compared to the control group.

Table 7: Effect of *Moringa oleifera* leaves on liver and kidney function of growing rabbits delivered from dams treated by Nanozinc, Nano-Moringa and *Moringa oleifera* leaves extract during gestation and lactation period:

Parameters	Treatment of dams								Significance					
	Control (NTD)		NZnD		NMoD		MoED		Treatment *vs.* control					
	Growing rabbits diets								NZnD		NMoD		MoED	
	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD
Total protein (g/dl)	4.37 ± 0.12	6.30 ± 1.17*	4.47 ± 0.20	5.83 ± 0.20	4.8 ± 0.12	5.63 ± 0.18	4.13 ± 0.45	5.30 ± 0.26	NS	NS	NS	NS	NS	NS
Albumin (g/dl)	3.07 ± 0.38	3.93 ± 0.12**	2.83 ± 0.19	4.97 ± 0.03**	2.60 ± 0.12	3.77 ± 0.33**	2.60 ± 0.26	4.47 ± 0.19**	NS	**	NS	NS	NS	NS
Globulin (g/dl)	1.30 ± 0.29	2.37 ± 0.07**	1.63 ± 0.24	0.87 ± 0.18	2.2 ± 0.23	1.87 ± 0.19	1.53 ± 0.19	0.83 ± 0.09	NS	*	NS	NS	NS	*
Cholesterol (mg/dl)	217.67 ± 2.91	80.67 ± 2.33**	123.00 ± 3.05	99.00 ± 1.53**	139.00 ± 2.89	67.00 ± 2.89**	96.33 ± 3.18	72.67 ± 2.33**	**	**	**	**	**	*
ALT (U/L)	19.44 ± 1.82	13.55 ± 1.82*	31 ± 2.65	35.89 ± 0.59*	40.33 ± 1.76	19.33 ± 0.33**	24 ± 1.53	24 ± 1.15	**	**	**	*	NS	**
AST (U/L)	20.2 ± 0.99	15.67 ± 1.76*	22.5 ± 0.29	28 ± 1.15**	30.89 ± 2.59	18 ± 0.58**	14 ± 1.15	25.55 ± 1.36**	NS	**	**	NS	**	**
Creatinine (mg/dl)	0.45 ± 0.07	0.56 ± 0.05	0.90 ± 0.07	0.66 ± 0.01**	0.66 ± 0.03	0.62 ± 0.04	0.41 ± 0.04	0.65 ± 0.01**	**	NS	**	NS	NS	NS

Data in table 8 showed that using MOL in growing rabbit rations raised the economic efficiency by increasing the overall return, net return, profitability, and relative profitability of rabbits' production.

Table 8: Economic efficiency of using *Moringa oleifera* leaves diet in growing rabbits delivered from dams treated by Nanozinc, Nano-Moringa and *Moringa oleifera* leaves extract during gestation and lactation period and those non treated dam.

Economic efficiency	Growing rabbits from NZnD,NMoD, MoED		Growing rabbits from NTD	
	BD	MD	BD	MD
Total feed intake(Kg/Rabbits)	35.630	34.925	10.325	12.040
Total weight gain (Kg/ Rabbits)	14.94	15.3	4.08	4.98
Price/kg feed(L.E)*	9.6	8.5	9.6	8.5
Total feed cost / Rabbits(L.E)	342	296.9	99.1	102.3
Price/kg live body weight(L.E)*	50	50	50	50
Total return/ Rabbits (L.E)	747	765	204	249
Net return(L.E)	405	468.1	104.9	146.7
Economic efficiency(EE)	1.18	1.58	1.06	1.43
Relative economic efficiency (REE)	100	133.9	100	134.9

*cost of feed /kg and price / kg live body weight was according to prices at September 2022.
NTD = Non Treated Dams NZnD = Nano-Zinc treated Dams NMoD = Nano-Moringa treated Dams. MoED = Moringa Extract treated Dams. BD = Basal Diet. MD = Moringa containing Diet.

DISCUSSION

Nano-sized minerals and plants are commonly used for improving animal reproduction and production. The effects of these nanoparticles may be extended to the neonates when they are supplemented to dams during pregnancy and lactation. For example, nanomaterials of Zinc oxide could transfer to neonates through the placenta and lactation **(Jo *et al*., 2013).** The results of the current study highlight the effect of giving dams nanoherbal supplements (nanozinc, nanoMOL, and MOL extract) during gestation and lactation on their growing rabbits' (GR) behaviour, in addition to the role of supplementing MOL to these GR on alleviating weaning stress with and without giving their dam the tested nanoherbal materials.

Data from the current study indicated that the MOL diet increased the feed intake and feeding behaviour frequency and duration of GR obtained from NTD and NZnD treated dams compared to GR fed on BD. However, there was no marked improvement in FCR, body weight, or gain of GR. Similarly, **El-Tazi (2014); Ramadan, (2017); El-Kholy *et al*., (2018)** noticed that feed intake and feeding behaviour increased significantly with a 5% moringa diet. Moreover, daily feed intake was increased in rabbits fed diets supplemented with 30 and 60 mg/kg Nano-ZnO **(Hassan *et al*., 2017)**. Meanwhile, **Selim *et al*., (2021)** stated that rabbits fed on diets containing 2.5, 5, and 7.5% of MOL showed no alterations in their feed intake. There is a lack of knowledge in the early literature about the role of MOL in pregnant dams on offspring behaviour and performance. MOL contains a phytosterol compound that has a lactogenic effect on dairy cows by increasing prolactin hormone **(Freeman *et al*., 2000)**. Similar to our findings, **Zendrato *et al*., (2019)** found no statistical change in FCR, weight, or weight gain in rabbits fed on MOL.

The obtained results revealed that giving zinc and MOL nanoparticles to pregnant does enhances feed intake and feeding behaviour in their GR. However, MOL diet decreased feed intake of GR from dams given these nanoparticles. Nanosized zinc was reported to improve the growth of weanling pigs due to enhanced digestibility, intestinal microbiota, gut integrity, and decreased oxidative stress **(Kim *et al*., 2022)**. Low doses of MOL increased feeding behaviour, unlike high MOL doses **(Teteh *et al*., 2013)**. Thus, this cumulative negative effect of MOL may explain the observed reduction in feeding of GR that was delivered from treated dams with MOL nanoparticles and moringa extracts and fed on a MOL containing diet.

The current study declared that MOL increased the drinking behaviour of GR. Similar findings were reported by **Abdella and Khalifah (2021)**; **El-Kashef, (2022)**. MOL has a potent diuretic effect **(Chukwuonye *et al*., 2013),** which leads to increased water loss and subsequently increased drinking behaviour. The latter outcome was correlated with an increase in blood albumin levels. The elevated albumin increased water viscosity, which enhanced animal motivation to drink. Moreover, **El-Desoky *et al*., (2017); El-Kashef (2022)** recorded 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 percent of the vascular colloidal osmotic pressure and plays a crucial role in maintaining tissue fluid homeostasis.

Our results showed that supplementing MOL either to dams or their GR (at the time of weaning) ameliorated the extensive self-grooming in relation to their BD groups. This observation was agreeable with those recorded by **Zade *et al*., (2013),** who found that various MOL extracts decreased non-genital grooming in male rats supplemented with MOL. On the other hand, treatment of dams with MOL extracts, zinc, and MO nanoparticles decreased the self-grooming behaviour of their GR. This effect was reversed when GR fed on MD. This result supports the hypothesis of a cumulative negative effect of prolonged MOL supplementation on rabbit performance and behaviour. Weaning usually poses stress on neonates, especially when it is accompanied by their separation from their littermates. Self-grooming is a behavioural indicator of anxiety and stress in rodents **(Song *et al*., 2016)**. Self-grooming duration increased under mild stress **(Fernández-Teruel *et al*., 2016)**. Hence, MOL could decrease weaning stress on the GR expressed by decreased self-grooming, which is correlated with an improvement in antioxidant activity. However, MOL could not mitigate weaning stress in GR from NZnD, NMoD, and MoED and fed on MOL. A combination of herbs and nano-sized minerals as an animal supplement may increase the required effects of both. However, some side effects may result, such as cumulative negative effects on GR. The antioxidant effect of the combination used could or could not face the resultant stress. In fact, Stress promotes the rate of lipid peroxidation, resulting in the enzyme glutathione peroxidase activity declining **(Schmitt *et al*., 2015)**.

Therefore, antioxidant activity in the current study declared that MOL increased the GSH level and reduced the blood MDA level in GR from NTD.

These observations are in accordance with **Selim *et al*., (2021)** who found that MOL decreased MDA at concentrations of 2.5, 5.0, and 7.5% of the diet. This case may be due to the potent antioxidant activity of MOL **(Yang *et al*., 2006)**. Additionally, MOL extract and powder reduced MDA levels along with reduced lipid peroxidation, hydroxyl-induced in rats, because of a direct antioxidant effect **(Serafini *et al*., 2011)**. **Kassem *et al*., (2022)** who stated that rabbits that received MOL extract increased GSH and lowered MDA. However, MOL GR from dams treated with NZn decreased GSH levels. **El Shemy *et al*., (2017)** noticed that rats administered 400 mg ZnO-NPs/kg body weight for seven days showed a significant decrease in GSH levels in the ZnO-NPs-intoxicated rats. On the other hand, Zinc is a crucial anti-stress mineral and promotes growth performance through its ability to produce antioxidant enzymes including GSH-Px and SOD **(Lee *et al*., 2022)**.

Moreover, MDA levels were reduced in GR from NZnD and fed on both BD and MD. The obtained data were in harmony with those of **Kamel *et al*., (2020)** who noticed that MDA concentrations were lower in the blood serum of rabbits treated with 30 and 50 mg ZnO/kg diets. This observation confirmed the role of zinc as an antioxidant defence system **(Farombi *et al*., 2004)** due to its high antioxidant effect **(Zheng *et al*., 2013)**. Thyroid hormones were altered in the tested treatment groups. MD increased the T3 level in GR compared to MoED and NZnD. Our data were in parallel with **El-Kashef, (2022)** who noticed that rabbits eating meals including MOL manifested the lowest concentrations of cortisol hormone and the highest concentrations of T3, when compared to the control group. The low cortisol level may be due to the potent antioxidant effects of MOL and zinc.

The obtained results indicated that MOL increased total protein levels in GR from NTD, and MOL also increased albumin levels in GR from all groups. MD elevated globulin in GR from NTD. These results were in consistency with **Omara *et al., (2018)*; El-Kashef, (2022)** who noticed that a diet containing 7.5% MOL increased total protein, albumin, and globulin compared with the control group. The high protein level could be due to the high nutritional value (higher methionine and lysine supply than soy beans) of the MOL. Moreover, MD reduced the total cholesterol of GR in all groups.

Similarly, **Salem *et al*., (2020)** noticed that the addition of MOL up to 20% in the diets of GR lowered serum total lipids, cholesterol, and triglycerides. The reduced cholesterol level of GR fed the MOL diets may be related to the phenolic compounds present in MOL **(Selim *et al*., 2021)**. On the other hand, **Etchu *et al*. (2017)** found no significant differences between the examined diet (10 and 20 % MOLM) and control groups for cholesterol. The levels of AST and ALT in serum are utilized to figure out if any body tissues, primarily the liver and heart, have been damaged **(Kasarala and Tillmann, 2016; Hasan *et al*., 2018).** MOL had marked hepatoprotective and cholesterol lowering effects **(Chukwuonye *et al*., 2013)**.

Our study data declared that MD-treated GR from NTD and NMoD decreased serum liver enzymes. Similarly, **El-Desoky *et al*., (2021); Selim *et al*., (2021)** found that supplementation of rabbits with MOL or nano-MOL reduced ALT and AST serum levels and indicated enhanced liver function. On the contrary, MD treated GR from NZnD and MoED showed elevated liver enzyme levels, and these data are augmented by **Mohamed *et al*., (2020)** who reported that prolonged administration of MOL increased liver enzyme levels that reflect liver damage. **Bayu *et al*., (2020)** found that chronic and acute toxic doses of MOL administration induce liver damage and enhance ALT, AST release in the serum of rats. In addition, **Ghareeb, (2021)** demonstrated that zinc oxide nanoparticles cause liver damage and consequently increase ALT and AST serum levels.

Our study revealed that MOL induced no alteration in creatinine levels. These findings suggest that the MOL has no negative effect on the proper functioning of the kidney **(Etchu *et al*., 2017)** because of its renal protective and diuretic effects **(Chukwuonye *et al*., 2013)**. In contrast, the creatinine level was increased in NZnD and MoED-GR (fed on MD). These results were similar to those of **Ismail and El-Araby, (2017)** who found that a diet containing 60 mg/kg Nano-ZnO increased plasma concentrations of creatinine in rabbits. The notable increase in creatinine level in our study may be due to the prolonged (prenatal and postnatal) treatment of GR by MOL **or a** nano-herbal combination causing renal toxic effects **(Oyagbemi *et al*., 2013)**.

The obtained results demonstrated that MOL supplementation for GR, whether treated prenatally or not showed an improvement in their economic efficiency, which is in agreement with **Alemede *et al*., (2014)** who observed a progressive decrease in the cost of feed by increasing the MOL replacement percent in the rabbits' diet. **Alain *et al*., (2016)** attributed the reduced feed cost to MOL replacement as it is rich in essential amino acid proteins and of low cost; consequently, MOL replacement in the diet decreased the feeding costs as MOL is cheaper than the soya bean**.**

CONCLUSION

Supplementing growing rabbits with MOL or pregnant and lactating rabbit does by MOL extract, nanozinc, and nanoMOL could improve rabbit health and behaviour without having a negative impact on the performance of the growing rabbits, in addition to increasing economic efficiency. However, supplementing growing rabbits (delivered from treated dams) with MOL induced stressful effects expressed by increasing self-grooming behaviour and oxidative stress in addition to renal and hepatic damage. Consequently, the obtained data does not recommend the use of MOL as a supplement for growing rabbits delivered from dams treated with herbal extracts or nanoherbal combinations. Further studies are highly needed to detect the accumulation of zinc in different tissues and muscles, in addition to the evaluation of the carcass quality of rabbits exposed to nano-herbal supplements.

Competing interes

There is no conflict of interests of any sort between authors or elsewhere.

REFERENCES

ABDELLA, M., and KHALIFAH, A., 2021. Effects of oral administration of *Lepidium sativum, Moringa oleifera* oils and aqueous extract of Vitex agnnus castus on reproductive performance and blood biochemical of Doe rabbits. Egyptian Journal of Rabbit Science, 31(1), pp.1-24. https://dx.doi.org/10.21608/ejrs.2021.127380

ABOU-ELKHAIR, R., SELIM, S., and HUSSEIN, E., 2018. Effect of supplementing layer hen diet with phytogenic feed additives on laying performance, egg quality, egg lipid peroxidation and blood biochemical constituents. Animal nutrition, 4(4), pp.394-400. https://doi.org/10.1016/j.aninu.2018.05.009

ALAIN MUNE MUNE, M., NYOBE, E.C., BAKWO BASSOGOG, C., and MINKA, S.R., 2016. A comparison on the nutritional quality of proteins from *Moringa oleifera* leaves and seeds. Cogent Food & Agriculture, *2*(1), p.1213618. https://doi.org/10.1080/23311932.2016.1213618

ALBRO, P.W., CORBETT, J.T., and SCHROEDER, J.L., 1986. Application of the thiobarbiturate assay to the measurement of lipid peroxidation products in microsomes. Journal of Biochemical and Biophysical Methods, 13(3), pp.185-194. https://doi.org/10.1016/0165-022X(86)90092-8

ALEMEDE, I.C., ONYEJI, E.A., TSADO, D.N., and SHIAWOYA, E.L., 2014. Reproductive response of rabbit does to diets containing varying levels of horseradish (Moringa oleifera) leaf meal. Journal of Biology, Agriculture and Healthcare, 4(19), pp.62-68. https://www.academia.edu/download/69037540/15210-17364-1-PB.pdf

ANSARI, S.H., ISLAM, F., and SAMEEM, M., 2012. Influence of nanotechnology on herbal drugs: A Review. Journal of advanced pharmaceutical technology & research, 3(3), p.142. https://doi.org/10.4103%2F2231-4040.101006

BAYU, F., AFEWORK, M., GELETA, B., ERGETE, W., and MAKONNEN, E., 2020. Effect of chronic administration of aqueous leaves extract of Moringa stenopetala on blood parameters and histology of liver and kidney in rats. Ethiopian Journal of Health Sciences, 30(2). https://doi.org/10.4314/ejhs.v30i2.14

BONIFACIO, B.V., DA SILVA, P.B., RAMOS, M.A.D.S., NEGRI, K.M.S., BAUAB, T.M., and CHORILLI, M., 2014. Nanotechnology-based drug delivery systems and herbal medicines: a review. International journal of nanomedicine, pp.1-15. https://doi.org/10.2147%2FIJN.S52634

CHUKWUONYE, I.I., CHUKU, A., JOHN, C., OHAGWU, K.A., IMOH, M.E., ISA, S.E., OGAH, O.S., and OVIASU, E., 2013. Prevalence of overweight and obesity in adult Nigerians–a systematic review. Diabetes, metabolic syndrome and obesity: targets and therapy, pp.43-47. https://www.tandfonline.com/doi/abs/10.2147/DMSO.S38626

DAL BOSCO, A., MUGNAI, C., MARTINO, M., SZENDRŐ, Z., MATTIOLI, S., CAMBIOTTI, V., CARTONI MANCINELLI, A., MOSCATI, L., and CASTELLINI, C., 2019. Housing rabbit does in a combi system with removable walls: effect on behaviour and reproductive performance. Animals, 9(8), p.528. https://doi.org/10.3390/ani9080528

DALLE ZOTTE, A., CELIA, C., and SZENDRŐ, Z., 2016. Herbs and spices inclusion as feedstuff or additive in growing rabbit diets and as additive in rabbit meat: A review. Livestock science, 189, pp.82-90. https://doi.org/10.1016/j.livsci.2016.04.024

DOUMAS, B.T., WATSON, W.A., and BIGGS, H.G., 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta, 31(1), pp.87-96. https://doi.org/10.1016/0009-8981(71)90365-2

EL SHEMY, M.A., AZAB, N.I., and SALIM, R.F., 2017. Zinc oxide nanoparticles: The hidden danger. injury, 2(45), p.61. http://dx.doi.org/10.11648/j.ijbbmb.20170201.11

EL-DESOKY, A., ALAZAB, A., BAKR, E., and ELSEADY, Y., 2018. Effect of adding moringa leaf meal to rabbit diets on some productive and reproductive performance traits. Egyptian Journal of Rabbit Science, 28(2), pp.263-286.

EL-DESOKY, N.I., HASHEM, N.M., ELKOMY, A., and ABO-ELEZZ, Z.R., 2017. Physiological response and semen quality of rabbit bucks supplemented with Moringa leaves ethanolic extract during summer season. Animal, 11(9), pp.1549-1557.

EL-DESOKY, N.I., HASHEM, N.M., GONZALEZ-BULNES, A., ELKOMY, A.G., and ABO-ELEZZ, Z.R., 2021. Effects of a nanoencapsulated moringa leaf ethanolic extract on the physiology, metabolism and reproductive performance of rabbit does during summer. Antioxidants, 10(8), p.1326. https://doi.org/10.3390/antiox10081326

EL-KASHEF, M.M. 2022. Impact of using moringa oleifera leaves meal in growing rabbit diets on productive performance, carcass traits and blood biochemical changes under heat-stress conditions. egyptian journal of rabbit science, 32(2), pp.141-162. https://doi.org/10.21608/ejrs.2022.267921

EL-KHOLY, K.H., BARAKAT, S.A., MORSY, W.A., ABDEL-MABOUD, K., SEIF-ELNASER, M.I., and GHAZAL, M.N., 2018. Effect of aqueous extract of Moringa oleifera leaves on some production performance and microbial ecology of the gastrointestinal tract in growing rabbits. Pak. J. Nutr, 17(1), pp.1-7. https://doi.org/10.3923/pjn.2018.1.7

ELLMAN GL. 1959. Arch Biochem Biophys 82:70–77

EL-RATEL, I.T., EL-KHOLY, K.H., MOUSA, N.A., and EL-SAID, E.A., 2023. Impacts of selenium nanoparticles and spirulina alga to alleviate the deleterious effects of heat stress on reproductive efficiency, oxidative capacity and immunity of doe rabbits. Animal Biotechnology, pp.1-14. https://doi.org/10.1080/10495398.2023.2168198

EL-TAZI, S.M. 2014. Effect of feeding different levels of Moringa oleifera leaf meal on the performance and carcass quality of broiler chicks. Int. J. Sci. Res, 3(5), pp.147-151. https://doi.org/10.1017/S1751731117000088

EMMANUEL, S.A., OLAJIDE, O.O., ABUBAKAR, S., IDOWU, I.D., ORISHADIPE, A.T., and THOMAS, S.A., 2014. Phytochemical and antimicrobial studies of methanol, ethyl acetate, and aqueous extracts of Moringa oleifera seeds. American Journal of Ethnomedicine, 1(5), pp.346-54.https://www.researchgate.net/publication/332528284

ETCHU, A.K., TIENTCHEU, B.L., GHOMSI, M.O.S., ENOW, J.T., TUEDOM, N.M., and ENAMOU, G., 2017. Effect of Moringa Oleifera leaf meal (Molm) on the growth, carcass, heamatology and biochemical parameters of Rabbits. SOJ Vet Sci, 3, pp.1-5. http://dx.doi.org/10.15226/2381-2907/3/3/00133

FAROMBI, E.O., HANSEN, M., RAVN-HAREN, G., MØLLER, P., and DRAGSTED, L.O., 2004. Commonly consumed and naturally occurring dietary substances affect biomarkers of oxidative stress and DNA damage in healthy rats. Food and Chemical Toxicology, 42(8), pp.1315-1322. https://doi.org/10.1016/j.fct.2004.03.009

FERNÁNDEZ-TERUEL, A., and ESTANISLAU, C., 2016. Meanings of self-grooming depend on an inverted U-shaped function with aversiveness. Nature Reviews Neuroscience, 17(9), pp.591-591. https://doi.org/10.1038/nrn.2016.102

FREEMAN, M.E., KANYICSKA, B., LERANT, A., and NAGY, G., 2000. Prolactin: structure, function, and regulation of secretion. Physiological reviews, 80(4), pp.1523-1631. https://doi.org/10.1152/physrev.2000.80.4.1523

GHAREEB, O.A. 2021. Toxicopathological effects of zinc oxide nanoparticles on the liver function and preventive role of silymarin in vivo. Indian Journal of Forensic Medicine & Toxicology, 15(2), pp.3212-3217. https://doi.org/10.37506/ijfmt.v15i2.14863

GOWRISHANKAR, R., KUMAR, M., MENON, V., DIVI, S.M., SARAVANAN, M., MAGUDAPATHY, P., PANIGRAHI, B.K., NAIR, K.G.M., and VENKATARAMANIAH, K., 2010. Trace element studies on Tinospora cordifolia (Menispermaceae), Ocimum sanctum (Lamiaceae), Moringa oleifera (Moringaceae), and Phyllanthus niruri (Euphorbiaceae) using PIXE. Biological trace element research, 133, pp.357-363. https://doi.org/10.1007/s12011-009-8439-1

HASAN, K.M.M., TAMANNA, N., and HAQUE, M.A., 2018. Biochemical and histopathological profiling of Wistar rat treated with Brassica napus as a supplementary feed. Food science and human wellness, 7(1), pp.77-82. https://doi.org/10.1016/j.fshw.2017.12.002

HASHEM, N.M., ABD EL-HADY, A., and HASSAN, O., 2013. Effect of vitamin E or propolis supplementation on semen quality, oxidative status and hemato-biochemical changes of rabbit bucks during hot season. Livestock Science, 157(2-3), pp.520-526. https://doi.org/10.1016/j.livsci.2013.09.003

HASSAN, F.A., MAHMOUD, R., and EL-ARABY, I.E., 2017. Growth performance, serum biochemical, economic evaluation and IL6 gene expression in growing rabbits fed diets supplemented with zinc nanoparticles. Zagazig Veterinary Journal, 45(3), pp.238-249. https://dx.doi.org/10.21608/zvjz.2017.7949

HENRY, R.J. 1964. Colorimetric determination of total protein. Clinical Chemistry. Harper and Row Publ., New York, USA, 181.

IGARASHI, E. 2008. Factors affecting toxicity and efficacy of polymeric nanomedicines. Toxicology and applied pharmacology, 229(1), pp.121-134. https://doi.org/10.1016/j.taap.2008.02.007

ISLAM, R., SULTANA, N., AYMAN, U., ISLAM, M.R., and HASHEM, M.A., 2022. Role of steroid growth promoter on growth performance and meat quality traits in broiler. Poultry Science, 101(7), p.101904. https://doi.org/10.1016/j.psj.2022.101904

ISMAIL, H.T.H., and EL-ARABY, I.E., 2017. Effect of dietary zinc oxide nanoparticles supplementation on biochemical, hematological and genotoxucity parameters in rabbits. Int. J. Curr. Adv. Res, 6, pp.2108-2115. https://www.researchgate.net/publication/323295205

JO, E., SEO, G., KWON, J.T., LEE, M., CHEUN LEE, B., EOM, I., KIM, P., and CHOI, K., 2013. Exposure to zinc oxide nanoparticles affects reproductive development and biodistribution in offspring rats. The Journal of toxicological sciences, 38(4), pp.525-530. https://doi.org/10.2131/jts.38.525

KAMEL, D.A., ABDEL-KHALEK, A.E., and GABR, S.A., 2020. Effect of dietary zinc-oxide or nano-zinc oxide on growth performance, oxidative stress, and immunity of growing rabbits under hot climate conditions. Journal of Animal and Poultry Production, 11(12), pp.565-571. https://dx.doi.org/10.21608/jappmu.2020.161193

KASARALA, G., and TILLMANN, H.L., 2016. Standard liver tests. Clinical liver disease, 8(1), p.13. https://doi.org/10.1002%2Fcld.562

KASSEM, M., ATWA, S., EL ZOGHBY, S.H.I.M.A.A., and KAMOURA, N.A., 2022. Protective role of aqueous moringa olifera leaves extract against adverse effect of cisplatin on hematoimmunobiochemical parameters in rabbits. Assiut Veterinary Medical Journal, 68(175), pp.39-48. https://doi.org/10.21608/avmj.2022.142731.1063

KIM, T., KIM, M., LEE, J., MOTURI, J., HA, S., TAJUDEEN, H., MUN, J., HOSSEINDOUST, A., and CHAE, B., 2022. Supplementation of nano-zinc in lower doses as an alternative to pharmacological doses of ZnO in weanling pigs. Journal of Animal Science and Technology, 64(1), p.70. https://doi.org/10.5187%2Fjast.2022.e2

LASHARI, M.H., TASLEEM, M.W., RAJPOOT, S.R., AFZAL, F., SHARIF, M., and AHMAD, M., 2021. Effect of Moringa Oleifera Leaf Extract on Ethogram of Rabbit Does. Annals of the Romanian Society for Cell Biology, 25(7), pp.1130-1141. https://www.researchgate.net/publication/356171392

LEE, J., HOSSEINDOUST, A., KIM, K., KIM, T., MUN, J., CHAE, B., and KIM, M., 2022. Improved growth performance, antioxidant status, digestive enzymes, nutrient digestibility and zinc bioavailability of broiler chickens with nano-sized hot-melt extruded zinc sulfate. Biological Trace Element Research, 200(3), pp.1321-1330. https://link.springer.com/article/10.1007/s12011-021-02747-6

MARTIN, P., BATESON, P.P. G., and BATESON, P., 1993. Measuring behaviour: an introductory guide. Cambridge University Press.

MAZZACHI, B.C., PEAKE, M.J., and EHRHARDT, V., 2000. Reference range and method comparison studies for enzymatic and Jaffe creatinine assays in plasma and serum and early morning urine. Clinical laboratory, 46(1-2), pp.53-55. https://www.researchgate.net/publication/12570319

MOHAMED, N.B., MOHAMED, A.H., ABU-AITA, N.A., NASR, S.M., NASSAR, S.A., and AHMED, K.A., 2020. Prophylactic role of moringa oleifera leaves' extract against lead toxicity in rabbits. Adv. Anim. Vet. Sci, 8(11), pp.1129-1141. http://dx.doi.org/10.17582/journal.aavs/2020/8.11.1129.1141

NATIONAL RESEARCH COUNCIL, 1977. Nutrient requirements of rabbits: 1977. National Academies Press.

NGOM, I., NGOM, B.D., SACKEY, J., and KHAMLICH, S., 2021. Biosynthesis of zinc oxide nanoparticles using extracts of Moringa Oleifera: Structural & optical properties. Materials Today: Proceedings, 36, pp.526-533. https://doi.org/10.1016/j.matpr.2020.05.323

OMARA, M.E., EL-ESAWY, G.S., RIAD, W.A., and MOHI EL-DIN, A.M.A., 2018. Effects of supplementing rabbit diets with Moringa oleifera dry leaves at different levels on their productive performance. Egyptian Journal of Nutrition and Feeds, 21(2), pp.443-453. https://dx.doi.org/10.21608/ejnf.2018.75584

OYAGBEMI, A.A., OMOBOWALE, T.O., AZEEZ, I.O., ABIOLA, J.O., ADEDOKUN, R.A., and NOTTIDGE, H.O., 2013. Toxicological evaluations of methanolic extract of Moringa oleifera leaves in liver and kidney of male Wistar rats. Journal of basic and clinical physiology and pharmacology, 24(4), pp.307-312. https://doi.org/10.1515/jbcpp-2012-0061

RAMADAN, S.G.A. 2017. Impact of supplementation of Moringa oleifera in diet of broiler chicks on their behaviour, welfare, performance and immune responses. Alexandria Journal of Veterinary Sciences, 55(1), pp.50-59. http://dx.doi.org/10.5455/ajvs.276323

RIFAI, N. 2017. Tietz textbook of clinical chemistry and molecular diagnostics-e-book. Elsevier Health Sciences

RÖSCHLAU, P., BERNT, E. and GRUBER, W., 1974. Cholesterol and esterified cholesterol. In Methods of enzymatic analysis (pp. 1890-1893). Academic Press.

SAALU, L.C., OSINUBI, A.A., AKINBAMI, A.A., YAMA, O.E., OYEWOPO, A.O., and ENAIBE, B.U., 2011. Moringa oleifera Lamarck (drustick) leaf extract modulates the evidences of hydroxyurea-induced testicular derangement. http://www.doaj.org/doaj?func=openurl&issn=19406223&genre=journal

SALEM, M.I., EL-SEBAI, A., ELNAGAR, S.A., EL-HADY, A., and MOHAMED, A., 2020. Evaluation of lipid profile, antioxidant and immunity statuses of rabbits fed Moringa oleifera leaves. Animal Bioscience. https://doi.org/10.5713/ajas.20.0499

SCHMITT, B., VICENZI, M., GARREL, C., and DENIS, F.M., 2015. Effects of N-acetylcysteine, oral glutathione (GSH) and a novel sublingual form of GSH on oxidative stress markers: A comparative crossover study. Redox Biology, 6, pp.198-205. https://doi.org/10.1016/j.redox.2015.07.012

SCHUMANN, G., and KLAUKE, R., 2003. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum:preliminary upper reference limits obtained in hospitalized subjects. Clinica chimica acta, 327(1-2), pp.69-79. https://doi.org/10.1016/S0009-8981(02)00341-8

SELIM, S., SELEIMAN, M.F., HASSAN, M.M., SALEH, A.A., and MOUSA, M.A., 2021. Impact of dietary supplementation with Moringa oleifera leaves on performance, meat characteristics, oxidative stability, and fatty acid profile in growing rabbits. Animals, 11(2), p.248. https://doi.org/10.3390/ani11020248

SERAFINI, M.R., DE LIMA, C.M., SANTOS, R.C., DÓRIA, G.A.A., DE JESUS, J.Y., DE MELO, M.G.D., DOS SANTOS, J.P.A., RABELO, T.K., FORTES, V.S., FONSECA, M.J.V., and DE ALBUQUERQUE JUNIOR, R.L.C., 2011. Pre-clinical toxicity of Morinda citrifolia Linn. leaf extract. African Journal of Biotechnology, 10(65), pp.14566-14572. https://doi.org/10.5897/AJB11.1388

SHARMA, V., SINGH, P., PANDEY, A.K., and DHAWAN, A., 2012. Induction of oxidative stress, DNA damage and apoptosis in mouse liver after sub-acute oral exposure to zinc oxide nanoparticles. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 745(1-2), pp.84-91. https://doi.org/10.1016/j.mrgentox.2011.12.009

SONG, C., BERRIDGE, K.C., and KALUEFF, A.V., 2016. 'Stressing'rodent self-grooming for neuroscience research. Nature Reviews Neuroscience, 17(9), pp.591-591. https://doi.org/10.1038/nrn.2016.102

TETEH, A., LAWSON, E., TONA, K., DECUYPERE, E., and GBEASSOR, M., 2013. Moringa oleifera leave: hydro-alcoholic extract and effects on growth performance of broilers. International Journal of Poultry Science, 12(7), pp.401-405. https://doi.org/10.3923/ijps.2013.401.405

YANG, R.Y., CHANG, L.C., HSU, J.C., WENG, B.B., PALADA, M.C., CHADHA, M.L., and LEVASSEUR, V., 2006. Nutritional and functional properties of Moringa leaves–From germplasm, to plant, to food, to health. Moringa leaves: Strategies, standards and markets for a better impact on nutrition in Africa. Moringanews, CDE, CTA, GFU. Paris, pp.1. https://www.researchgate.net/publication/242174126

ZADE, V., DABHADKAR, D., THAKARE, V., and PARE, S., 2013. Evaluation of potential aphrodisiac activity of Moringa oleifera seed in male albino rats. Int J Pharm Pharm Sci, 5(4), pp.683-689. https://www.researchgate.net/publication/286135159

ZENDRATO, D.P., GINTING, R., SIREGAR, D.J.S., PUTRA, A., SEMBIRING, I., GINTING, J., and HENUK, Y.L., 2019, Growth performance of weaner rabbits fed dried Moringa oleifera leaf meal. In IOP Conference Series: Earth and Environmental Science (Vol. 260, No. 1, p. 012058). IOP Publishing. http://dx.doi.org/10.1088/1755-1315/260/1/012058

ZHENG, J., ZHANG, Y., XU, W., LUO, Y., HAO, J., SHEN, X.L., YANG, X., LI, X., and HUANG, K., 2013. Zinc protects HepG2 cells against the oxidative damage and DNA damage induced by ochratoxin A. Toxicology and applied pharmacology, 268(2), pp.123-131. https://doi.org/10.1016/j.taap.2013.01.021

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