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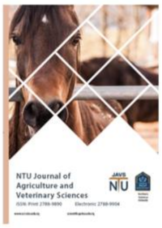

Effect of adding two types of zinc oxide to the ration on body weight, milk yield and milk components of Awassi ewes during lactation season

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A B S T R A C T

This experiment was carried out in animal field of Kosar Com./Erbil, to study the effect of zinc oxide ZnO or nano Zinc oxide nZnO particles as feed supplementation on productive performance and milk components of Awassi ewes during the three months of lactation. 30 pregnant Awassi ewes 30 days before parturition, aged 3-4 years with an average weight of 58 ± 2.19 kg were used. Ewes were randomly distributed into three groups (10 ewes / group). Basal ration were given (concentrated mixture + hay), the first group was considered a control group fed basal ration, second group was fed on basal ration + 60 mg/DM zinc oxide, third group was fed on basal ration + 60 mg/DM zinc oxide nanoparticles. Daily milk production from ewes was recorded and its components were measured biweekly and the monthly rate was calculated. Results indicate no significant differences in daily weight and dry matter intake in treatment groups compared to control. Milk production and daily milk yield were significant ($p \leq 0.05$) higher on the nZnO treatment compared to control group during the second and third months of lactation, and a significant ($p \leq 0.05$) increase in percentage and milk fat yield during the three lactation months. Moreover nZnO treatment showed a significant ($p \leq 0.05$) superiority in the amount of milk protein at the first and third month of lactation, and lactose at the third month of lactation. Also, nZnO treatment was significantly ($p \leq 0.05$) superior in Zn concentration in milk compared to the control at the second and third month of lactation. The result revealed that zinc oxide nanoparticles as dietary supplements improved the amount and some components of milk in domestic Awassi ewes .

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Introduction

Recent studies focus on the use of non-traditional feed additives in animal feed in order to improve their nutritional value quantitatively and qualitatively to reach the best level of productivity, and these methods include the use of trace mineral elements such as iron, selenium, chromium, cobalt and zinc as additives to animal feed, which the body needs in small quantities and cannot be stored inside the body, which has proved its need and its important role in increasing the growth rate and production and raising the level of immunity in farm animals [1] [2] [3]. Mineral elements also play an important role in animal nutrition, affecting various physiological functions and overall productivity, and a proper mineral balance is necessary to maintain animal health and reproductive efficiency [4] [5] [6].

Zinc is one of the most important essential trace minerals that cannot be synthesized by the body, it is an important component and the work of about 300 enzymes of the body that work to regulate many physiological processes in the body, and it is necessary and vital for cell building, protein synthesis and tissue growth, It also acts to strengthen the body's immune system, the deposition of minerals in the bones, has an effect on the function of the thyroid gland, zinc also enters into the synthesis of deoxyribonucliec acid (DNA), ribonucliec acid (RNA), cell division and growth of embryos during pregnancy and during the lactation period [7; 8; 9]. In addition to the antioxidant properties of zinc and its role in reducing oxidative stress [10]. Also, Zinc plays an important role in enhancing productive qualities in various farm animals, and this is evidenced by many studies, as zinc supplements have a role in improving growth performance and reproductive qualities in livestock, and this indicates the fundamental role of zinc in metabolic processes and general health [11] and its importance in reproductive performance and fertility improvement[12].

Zinc oxide nanoparticles (ZnO NPs) is one of the new metal elements that have been newly produced and marketed using nanotechnologies. Zinc oxide nanoparticles have attracted a lot of attention due to their distinctive properties of small size, increased surface to volume ratio of nanoparticles and wide variety of applications [13]. ZnO NPs, which are produced using a variety of technologies including physical, chemical and biological processes [14; 15]. This material ZnO NPs has been used in many scientific fields such as dyes, food, electronics, as well as in medicine [16; 17; 18]. In addition, recent studies of researchers have focused on studying the impact of zinc and zinc nanoparticles on the productive performance of the animal of milk and its components, in view of the benefits of sheep's milk and as an increase in demand for it resulting from the increase in the population. So, It has become necessary to apply all kinds of strategies and efforts to improve the quality and quantity of milk as well as maintain the udder health of milk animals, as the researchers focus on the factors affecting milk production and the use of modern technologies to enhance its production [19] [20].The researchers observed an effect of zinc on milk production when using zinc and zinc nanoparticles as dietary supplements and obtained a significant increase in the production and quality of milk [21] [22] [23].

Therefore, this experiment was taken up to studying the effect of addition zinc oxide particles or Zinc oxide nanoparticle as dietary supplements on the production performance and quantity and quality of milk yield during lactation months in domestic Awassi ewes.

Material and methods

This study was conducted in the animal field of Kosar company/ Erbil in cooperation with the Department of animal production / Faculty of Agriculture and Forestry / University of Mosul. In this experiment, 30 Awassi ewes aged 3-4 years with an average weight of 58 ± 2.19 kg were utilized in this study, and the ewes were in the final stages of pregnancy 30 days prior to their expected parturition. Pregnant ewes were selected from the herd in the field after pregnancy screening using an ultrasound machine. The ewes were housed in a semi-open type barn with an area of (4 × 5) M., and clean water was provided ad-libitum throughout the experimental period . All ewes were maintained on basal diet consisting of (concentrate mixture + wheat hay) as the ration was provided on the basis of 2.5% of live body weight and its ingredients are installed in Table (1).

The ewes were randomly divided into three groups (10ewes/group): the control group served basal ration without any addition, second group served basal ration + 60 mg /kg DM zinc oxide ZnO, third group served basal ration + 60 mg/kg DM zinc oxide nanoparticles nZnO. The two forms of ZnO particles were mixed with concentrate feed mixture over and above available from basal ration (11.71mg) Diet was served on two meals a day at 8am and 4pm for the duration of the experiment, and the remaining amount of feed was recorded on the morning of the second day. In addition to providing mineral salt blocks and fresh water to the animals continuously throughout the experiment period, in addition, the ewes went out to graze daily in the pastures of the field. Throughout the experiment, the animals underwent health care to ensure their safety from diseases and according to the preventive program followed in the company's field. The experimental data were taken during the 3-month of lactation period.

Table 1. Ingredient and chemical composition of basal ration

Ingredients	Percentage %
Barley	64
Wheat bran	23.5
Soya bean meal	5
Wheat straw	5
Limestone	1
Salt	1
Urea	0.5
Chemical composition **	
Dry Mater	90.46
Organic Mater	92.75
Crude Protein	14.6
Ether Extract	4.32
Crude Fibers	9.12
Met. Energy, Kcal./kg	2650
Zinc mg/kg DM ***	11.71

* Ingredient of diet were taken from NRC [24].
** Chemical composition calculated according to (A.O.A.C.[25]
Met. energy of diet was calculated according to Alkhawaja [26]
*** Zinc concentration calculated according to (Chapman and pratt, 1961)[27]

Source of zinc

Zinc oxide and zinc nano-oxide were purchase from local markets from the Office of Arij al-Furat located in Bab al-Muazzam /Baghdad, American origin of the company SIGMA CHEMICAL COMPANY , The necessary tests were carried out on the zinc nanoparticle material at the AL-Khora Laboratory in Baghdad to find out the effectiveness of the material

Studied qualities

Animal weights and the amount of feed intake Live body weight LBW of ewes were calculated at the end of each month of the experiment using a special (EALTE) type scale English made weighing (150)kg.

. The remaining feed from all the animals of each group was estimated separately using a dedicated feed balance to calculate the amount of dry matter intake DMI per day and this process continued during the experimental period .

Milk production

Manual milking was used for milking ewes twice a day at 8 am and 4 pm, and when estimating the daily milk production, the newborns were isolated from their mothers for 12 hours, then the ewes were milked the next morning and the production of one milking for ewe was multiplied by 2. Milk production measurements were taken biweekly and milk production was recorded for each month until the lambs were weaned (3 months).

Milk components

The proportions of milk components were measured using the German-origin milk analyzer, samples were taken immediately after milking by 50 ml milk sample per ewe. Sample was placed in device and after the analysis process has started with a minute and a half, the results appear on the device screen as percentages of each component of milk, which includes fat, solids non-fat SNF, density, protein, lactose sugar, and the measurement was done fortnightly during the lactation months until the age of weaning.

Zinc estimating in milk

Zinc ZN concentration in the milk samples was estimated by burning the samples and subsequently digesting the milk samples using wet digestion using concentrated sulfuric acid H2SO4 and perchloric acid HCLO4 . 0.5 ml of the milk sample was taken and then 10 ml of sulfuric acid was added to it, left for 24 hours, after which the samples were placed in the oven with the addition of drops of hydrochloric acid, the extract was quantitatively transferred to a volumetric flask of 50 ml and distilled water was added to it to the mark and the samples are measured directly according to (Chapman and pratt, 1661).

Statistical analysis

The statistical analysis was carried out using Complete Randomized Design (C.R.D) with a simple experiment, and Duncan multi-range test was used to calculate stander errors and the differences between groups at $p \leq 0.05$ [28], mediated by the ready-made statistical analysis program SAS, [29].

Results

Effect of dietary additioning of zinc oxide and nano zinc oxide on productive performance, milk yield and its components for Awassi ewes at the first month of lactation.

Results in Table (2) indicated the absence of a significant effect of addition 60 mg/kg DM ZnO and nZnO particles in amount of dry matter ntake DMI, body weight BW of ewes, daily and total milk yield in treatment groups compared to control group in the first month of lactation.

As for the milk components, a significant ($p \leq 0.05$) increase was observed in the percentage of milk fat for the group dietary supplemented with nZnO than the group supplemented with ZnO, which in turn significantly ($p \leq 0.05$) outperform the control group. On the other hand, only nZnO treatment outperformed the control group in both the amount of fat in milk, amount of protein and amount of solids not fat SNF. While no significant effect of ZnO treatments observed in other milk conponents (protien %, lactose %, SNF %, milk density %), in addtion to insignificant increase in Zinc concentration in milk.

Effect of dietary additioning of Zinc oxide and nano Zinc oxide on productive performance, milk yield and its components for Awassi ewes at the second month of lactation

The results in Table (3) showed that there was no significant effect of adding nZnO and ZnO on the amount of DMI and BW of ewes in the treated groups compared to the control in the second month of lactation, The results also showed a significant ($p \leq 0.05$) superiority of the nZnO treatment only over control group in daily milk yield, total milk yield, milk fat percentage, the amount of milk fat, protien, lactose and SNF.While the treatment groups supplemented with two forms of ZnO showed no significant effect on protein, lactose, SNF and milk density percentages compared to control group. On the other hand, the result revealed a significant increase in Zinc concentration in milk in both treated groups compared to control.

Effect of dietary additioning of Zinc oxide and nano Zinc oxide on productive performance, milk yield and its components for Awassi ewes at the third month of lactation.

The results in Table (4) indicated that there was no significant effect of supplementing nZnO or ZnO on the amount of feed intake and live body weights of ewes in treated groups compared to control in the third month of lactation. On the other hand results showed a significant increase($p \leq 0.05$) in the amount of daily milk of ewes supplemented with nZnO compared to ZnO and control groups. As well as results also showed a significant ($p \leq 0.05$) effect only for nZnO treatment compared to control in total milk yield and the amount of milk fat, protein, lactose and SNF. While there was no significant effect of the treatments on the percentage of protein, lactose, SNF and milk density compared to control. In contrast there is a significant ($p \leq 0.05$) increase in Zn concentration in milk.

Discussion

The results of the study indicated that there was no significant effect of supplementing nZnO or ZnO on the amount of DMI and BW in the experimental treatments during three month of lactation , and these results agreed with [30; 7; 23] .who did not obtain a significant differences in DMI and BW in milking ewes treated with various forms of Nano and non-nano Zinc particles as feed supplements. But the results did not agree with [31] who found a significant increase in DMI in ewes supplemented with ZnO NPs compared to control group. Previous studies on the effect of using zinc as a dietary supplement on milk production were variable. Our results about milk yield and quality in first month of lactation showed that there was no significant effect on the amount of milk yield from ewes treated with two forms of zinc compared to the untreated group. The results agreed with [23] and [32], who did not obtained a significant effect of zinc and zinc nanoparticles on milk yield produced from ewes or cows during the first month of lactation. As well as, there were no significant effect of zinc treatments on percentage of protein, lactose, SNF and milk density. These results agreed with [30] and [7], who did not obtain a significant effect on the percentages of lactose, SNF and milk density in ewes milk treated with nZnO or different source of zinc compared to control group during the first month of lactation. Nevertheless our study showed a significant increase in the percentage and amount of milk fat, protein and SNF yields in the ewes supplemented with nZnO or ZnO compared to control group. These results inconsistent with [23] who did not obtained a significant effect of dietary supplementing with 60 mg/head nZnO and ZnO in the percentage of milk fat. Moreover, our result did not agree with them, which they obtained a significant increase in the percentage of milk protein and lactose among the group treated with nZnO and other treatments groups during this month. Results of our study Tables (3 , 4) indicated that the amount of total and daily milk yield in all treatments increased with the progressive of lactation period, and this consistent with [32],who stated that milk production gradually increases with the progressive of lactation. So increases in milk yield were obtained from both treatment groups with ZnO , especially in nZnO treatment which obtained an a significant increase in milk yield compared to control group during the second and third months of lactation. The results agreed with [7] and [23], who obtained the highest milk yield in nZnO and ZnO treatments compared to control group during the second month of lactation. As for the milk components, the results were consistent with the above researchers in that they did not have a significant difference in the percentage of protein, lactose, non-fat solids and density of milk in the treatment supplemented with nZnO compared to control during the second month of lactation , While the results did not agree with above researchers that they did not get a significant difference in the percentage of milk fat. Moreover, the results about the third month of lactation agreed with [21; 31 and 32],who indicated the advantage of dietray supplementing with nZnO in increasing milk yeild in ewes compared to the control group during this month, while our results in contrast with [33] and [34], who did not obtained a significant effect of using different forms of nano zinc and non-nano Zn particles on milk production of cows. As for the milk components , our results in agreement with [33] and [23] who obtained a high significant percentage of milk fat, and non-significant differences in the percentages of protein, lactose, SNF and milk density in nano and non- nano zinc oxide particles treatments compared to control group. While the results of our study inconsistent with [31] who did not found any differences in milk components of ewes treated with nZnO or ZnO particles. The increase in milk production and some of its components during lactation months in ewes that were fed on 60 mg /kg ZnO and nZnO may be due to the fact that zinc element led to improved food digestion, increased activity of digestive enzymes and thus increased feed consumption by the animal, and the use of zinc or nano zinc oxide in the diet leads to improved growth of rumen bacteria and an increase in the efficiency of energy utilization [35], in addition to the role of zinc oxide in increasing vital activity and as a protective substance that kills pathogenic bacteria and preserves the epithelium of the mammary glands, which affects on milk production [32]. Also, Zinc of all forms is one of the elements that enter into the synthesis and increase the activity of enzymes responsible for the reproduction and division of udder cells and thus improve the condition and health of the udder, which leads to increased milk production [22], in addition to the role of zinc in increasing the secretion of the hormone prolactin, which acts on the initiation and continuation of milk secretion [36]. As for the high concentration of zinc in ewes milk feeders on nZnO, it should be noted that the milk content of minerals especially microelements depends on there content in the feed [37]. That is why it is so important to use materials with high biological activity, which may be nanometals. Recently, work has been carried out on the possibility of improving the quantity and quality of milk using nanometallics, and by influencing the patterns of oxidative stress harmful to milk tissues and the possibility of adding zinc oxide nanoparticles as dietary supplements and increasing zinc in milk efficiently without affecting on the quality of milk. The results indicated that the concentrations of zinc in the milk of ewes fed on zinc oxide nanoparticles or zinc oxide indicated that the ratio of zinc transferred to milk was similar and differed only significantly than control group, noting that the values of zinc concentrations in milk of experimental treatments during the months of lactation did not exceed the values (3.8-5) mg / l recommended by the food and Agriculture Organization / WHO/FAO [38].The high level of zinc concentration in ewes milk treated with nZnO is probably due to the fact that the biological activity of zinc oxide nanoparticles is higher than zinc or zinc oxide particles [34], and may be due to an increase in feed consumption and thus an increase in zinc consumption, high concentration of zinc in the blood plasma , and an increase in the transfer of zinc from blood to milk. The results agreed with [22] who obtained a significant increase in the zinc concentration in the milk of cows given 40 mg / kg feed of nZnO as dietary supplements compared to the control group , While the results did not agree with [33], who did not obtain a significant difference in the level of zinc concentration in the milk of cows supplemented with 60 mg/ kg feed of nano and non-nano zinc particles.

Conclusion

Through the results of this study, it can be concluded that dietary supplementation with zinc oxide, especially nano zinc oxide, showed significant improvement in milk yield in Awassi ewes, in addition to its role in significant improving the milk components of fat and protein, especially in the third month of lactation and increased in Zn concentration in milk without exerting any negative effects on milk yield and composition, as well as their health status. Thus, nZnO supplementation can be adopted in ewes feeding and may potentially serve as an alternative zinc source with high bioavailability.

CONFLICT OF INTEREST:

The authors reported no conflict of interest.

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Table 2. Effect of dietary supplementation of nZnO and ZnO on body weight, milk yield and its components of Awassi ewes at the first month of lactation.

Traits	Treatments		
	Control	ZnO (60mg)	nZnO(60mg)
Dry Mater Intake (kg/d)	1.308± 51.55 a	1.314±48.43 a	1.324±27.19 a
Body Weight (kg)	52± 3.54 a	51.8± 5.02 a	52.08±3.54 a
Daily milk yield (g/d)	233.33± 57.74 a	266.67± 115.47 a	333.33± 57.74 a
Total milk yield (kg/d)	7.00± 1.73 a	8.00± 3.46 a	10.00± 1.73 a
Fat %	4.64± 0.30 c	5.62± 0.44 b	6.53±0.26 a
Protein %	4.17± 0.08 a	4.28±0.60 a	4.47± 0.36 a
Lactose %	4.19± 0.23 a	4.04±0.56 a	4.23± 0.32 a
SNF %	8.07±0.71 a	8.46± 1.33 a	9.22± 0.53 a
Fat (g/d)	10.91± 3.24 b	14.76± 5.51 ab	21.76±3.75 a
Protein (g/d)	9.70± 2.20 b	10.96± 2.99 ab	14.78±1.39 a
Lactose (g/d)	9.05±2.24 a	10.35± 4.48 a	12.93± 2.24 a
SNF (g/d)	18.69± 3.83 b	21.99±7.58 ab	30.54±3.38 a
Milk density %	44.68± 23.00 a	29.39± 8.00 a	30.13±5.31 a
Zinc conc. (mg/l)	1.28±0.17 a	1.47± 0.55 a	1.54± 0.23 a

a,b,c Within a row, means without a common superscript letter differ with $P \leq 0.05$

Table 3. Effect of dietary supplementation of nZnO and ZnO on the body weight, milk yield and its components of Awassi ewes at the second month of lactation.

Traits	Treatments		
	Control	ZnO (60mg)	nZnO(60mg)
Dry Mater Intake (kg/d)	320.1 ± 24.12 a	1.332± 33.20 a	1.335 ± 46.32 a
Body Weight (kg)	54.4±2.07 a	55.0±5.92 a	54.4±1.82 a
Daily milk yield (g/d)	366.67±76.38 b	460±163.71 ab	640±69.28 a
Total milk yield (kg/d)	10.50±2.60 b	13.80±4.91 ab	19.20±2.08 a
Fat %	5.58±0.44 b	6.14±0.44 ab	6.62±0.18 a
Protein %	4.36±0.17 a	4.68±0.50 a	4.51±0.40 a
Lactose %	4.13±0.14 a	4.48±0.41 a	4.30±0.47 a
SNF %	9.27±0.34 a	9.63±1.42 a	9.69±0.83 a
Fat (g/d)	20.36±3.73 b	28.73±12.25 ab	42.33±4.18 a
Protein (g/d)	15.91±2.79 b	21.91±9.89 ab	29.06±5.80 a
Lactose (g/d)	15.09±2.67 b	20.94±9.27 ab	27.68±5.70 a
SNF (g/d)	33.81±5.77 b	45.18±21.55 ab	62.34±11.61 a
Milk density %	31.44±1.53 a	35.54±2.60 a	31.41±3.54 a
Zinc conc. (mg/l)	1.31±0.05 b	1.56±0.14 a	1.67±0.08 a

a,b, Within a row, means without a common superscript letter differ with *P* ≤0.05

Table 4. Effect of dietary supplementation of nZnO and ZnO on thebody weight, milk yield and its components of Awassi ewes at the third month of lactation.

Traits	Treatments		
	Control	ZnO (60mg)	nZnO(60mg)
Dry Mater Intake (kg/d)	1.330± 35.33 a	1.335 ±44.32 a	1.340 ±51.12 a
Body Weight (kg)	56.8±1.92 a	57.0±5.43 a	56.8±1.92 a
Daily milk yield (g/d)	466.67±47.26 b	565.00±108.28 b	690.00±65.57 a
Total milk yield (kg/d)	14.00±1.42 b	16.95±3.25 ab	20.70±1.97 a
Fat %	3.14±0.23 b	3.54±0.23 b	5.08±1.21 a
Protein %	4.89±0.50 a	5.03±0.41 a	5.13±0.24 a
Lactose %	4.60±0.20 a	4.85±0.27 a	4.76±0.08 a
SNF %	10.65±0.51 a	10.68±0.65 a	10.64±0.22 a
Fat (g/d)	14.63±1.52 b	20.11±4.78 b	35.50±11.59 a
Protein (g/d)	22.90±4.12 b	28.30±4.73 ab	35.46±4.24 a
Lactose (g/d)	21.41±1.28 b	27.33±4.94 ab	32.88±3.62 a
SNF (g/d)	49.81±7.03 b	60.30±11.68 ab	73.47±8.16 a
Milk density %	40.20±1.22 a	40.41±1.69 a	38.87±0.44 a
Zinc conc. (mg/l)	1.34±0.12 c	1.70±0.18 b	2.15±0.11 a

a,b,c Within a row, means without a common superscript letter differ with *P* ≤0.05