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Short communication

Ultrasound changes in meat yield of shami goats (*Capra aegagrus hircus*) fed diet supplemented with zinc oxide nanoparticles

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ABSTRACT

The higher bioavailability, lower toxicity, and lower potential environmental risks of zinc oxide nanoparticles (ZnO NPs) compared to traditional sources of zinc (Zn) encouraged researchers to use it in animal diets. The current study aimed to investigate the effects of dietary low ZnO NPs levels supplementation on the meat yield of Shami goats using ultrasound examination. A total of thirty healthy female Shami goats with an average body weight of 38.43 ± 1.94 kg at 2–3 years old were randomly distributed into three equal groups (10 goats/group). The first group was fed a basal diet (control), while the second and third groups were fed a basal diet supplemented with 11.25 mg/kg (T1) and 22.50 mg/kg (T2) dry matter (DM) of ZnO NPs, respectively, for 12 weeks. *Longissimus dorsi* muscle ultrasound evaluation at both the thoracic and lumbar regions revealed denser transverse process-related acoustic shadowing (TS), rib-related acoustic shadowing (RS), and muscular shadowing (MS) in goats supplemented with ZnO NPs, T1 and T2, compared to goats fed a control diet. Furthermore, ultrasound measurements of the thoracic and lumbar *longissimus dorsi* regions of goats supplemented with ZnO NPs at both levels revealed greater fat thickness, depth, perimeter, and area compared to animals fed a control diet ($p < 0.05$). This improved trend was dose-dependent, with T2 values being higher than T1 values ($p < 0.05$). In conclusion, the ultrasound assessment results showed that supplementing Shami goats with ZnO NPs at these two low levels enhanced meat yield.

1. Introduction

The higher ability of goats to adapt to the diverse production systems enables them to become well-adapted widespread species (Guerrero et al., 2018). A dual-purpose breed (*i.e.*, Damascus or Shami) of goat has been given priority by the Food and Agriculture Organization (Al-Saef, 2013) and successfully distributed to more than fifteen Middle East Countries (Mavrogenis et al., 2006).

Zinc (Zn) plays a pivotal role as a trace mineral in the main biological functions (Mohd Yusof et al., 2019; Suttle, 2010). It is well established that, Zn promotes growth (Liu et al., 2011; Swain et al., 2016), improves performance (Fadayifar et al., 2012), acts as a powerful antioxidant (Jiang et al., 2018) by preventing lipid peroxidation (Sloup et al., 2017), and improves the immune function (Song et al., 2021). Zn can also alter the intramuscular fat content of breast meat (Liu et al., 2011) possibly by modulating the gene expression of fat metabolism-related enzymes in broilers (Liu et al., 2015).

Ruminants only absorb a portion of the minerals they digest,

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including Zn, so their dietary requirements vary considerably from their net requirements. This is due to the fact that ruminant diets high in grains allow for the escape of partially digested or undegraded phytate (Suttle, 2010). Indeed, the undigested phytate will ultimately interfere with mineral absorption (Leytem et al., 2007), reflecting the lower availability of minerals. Thus, supplementation is necessary to meet animal's requirements (Bravo et al., 2003; Kincaid et al., 2005). Zn is usually supplemented to the diet of animals in the form of inorganic or organic sources (Swain et al., 2016). The bioavailability of inorganic forms are substantially lower than that of organic forms while the use of organic chelates of Zn is limited due to the higher cost (Zhao et al., 2014). However, high levels of Zn supplementation for animals would increase the risk of toxicity or pose an environmental concern (Feng et al., 2009), because fecal nutrient contents are a direct reflection of dietary nutrient contents (Oryschak, 2005). Notably, Zn in the form of nanoparticle (NP) has a larger surface area of the NP granules which results in higher bioavailability and therefore an efficient absorption (Mohd Yusof et al., 2019; Swain et al., 2016)

The ability to accurately determine body or carcass composition is substantially important for measuring, grading, choosing, and ultimately purchasing meat-producing animals. Real-time ultrasound examination is the most recent advanced non-invasive technique for collecting data on meat carcass composition of farm animals under field conditions (Scholz et al., 2015). These advantageous properties, as well as previous research, suggested that ultrasound could be used as a simple routine evaluation method in farms to predict meat yield (Grill et al., 2015).

We hypothesized that supplementation of ZnO NPs to diets of Shami goats at relatively lower doses could be bioavailable, highly effective, non-toxic, and less expensive, and could improve the meat yield. Therefore, the present study aimed to investigate the effect of dietary low ZnO NPs levels supplementation, 11.25 and 22.50 mg/kg DM, on the meat yield of Shami goats in both the thoracic and lumbar regions of the *longissimus dorsi* (eye) muscle using a non-invasive ultrasound scanning approach.

2. Materials and methods

2.1. Preparation and characterization of ZnO NPs

The powder of ZnO NPs was synthesized in our laboratory using a solid-state method following the procedure previously described by El-Bahr et al. (2020). The particle size of the resulting ZnO NPs was examined using a high-resolution transmission electron microscope (JEM-1200, JEOL, Japan) and the morphological features of the synthesized ZnO NPs were explored using field emission scanning electron microscopy (Quanta FEG 250, FEI, USA) as described by El-Bahr et al. (2020).

2.2. Animals, management, and diets

All procedures and experimental protocols as well as management conditions followed in this feeding trial were carried out following the Guidelines for the Care and Use of Agricultural Animals in Research and Teaching, Federation of Animal Science Societies (FASS, 2010). Besides that, the Institutional Animals Care and Use Research Ethics Committee of the University of Benha, Faculty of Veterinary Medicine, also approved the current feeding trial (BUFVTM, 02102018, 02 October 2018).

Thirty healthy female Shami goats, aged 2–3 years old, with an average body weight of 38.43 ± 1.94 kg were used for this feeding trial at the farm station facility of the Faculty of Agriculture, Benha University of Egypt. All goats were kept in roof-pens separated by wooden partitions in a confined system under the same environmental, managerial, and hygienic conditions. They were maintained in ambient temperature and natural daytime and light throughout the whole period of the experiment during the spring season. Goats were randomly distributed into three equal groups (10 goats/group). The goats were fed the following diets: control (basal diet without ZnO NPs), T1 and T2, which were basal diets supplemented with 11.25 and 22.50 mg ZnO NPs/kg DM diet, respectively. Experimental diets were formulated as a total mixed ration (TMR) to meet the optimum nutrient requirements of goats as recommended by NRC (2007). The ingredient and chemical composition of the concentrate mixture (CM) and TMR of the experimental diets and nutrient specifications of the basal diet are shown in Table 1. The required amounts of ZnO NPs were dissolved in three equal volumes of distilled water (w/v) using Branson 2200 ultrasonic cleaner water bath (B2200R-4 Laboratory Sonic Digital, Branson Ultrasonics, Sigma Aldrich, St. Louis, MO 63178, USA). Fully dissolved ZnO NPs in distilled water were sprayed while mixing with TMR to precisely obtain the three desired concentrations of the experimental diets. The TMR has been prepared and mixed on a fresh daily basis. Goats were kept under the experimental protocol from 1st to 12th-week post-kidding and offered both feed and water *ad libitum*.

Proximate analysis of the basal diet for dry matter (DM), ash, crude protein (CP), and crude fat (EE) was conducted according to the standard methods of AOAC (2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined following the procedure described by Van Soest et al. (1991). Zn content

Table 1
Ingredients and chemical composition of the concentrate mixture (CM) and total mixed ration (TMR) of the experimental diets (g/kg dry matter, DM, unless otherwise indicated).

	[a]Control	[b]T1	[c]T2
Ingredients composition of concentrate mixture (CM) (g/kg dry matter, DM)			
Cotton seed meal (41 % [d]CP)	350.00	350.00	350.00
Wheat bran	330.00	330.00	330.00
Corn, yellow (8 % CP)	222.00	222.00	222.00
Rice bran	40.00	40.00	40.00
Molasses	30.00	30.00	30.00
Calcium carbonate	20.00	20.00	20.00
Sodium chloride	5.00	5.00	5.00
[l]Vitamin and mineral mixture	3.00	3.00	3.00
Ingredients composition of total mixed ration (TMR) (g/kg dry matter, DM)			
Concentrate mixture (CM)	401.60	401.60	401.60
Egyptian clover	287.40	287.40	287.40
Corn silage	98.40	98.40	98.40
Wheat straw	212.60	212.59	212.58
Zinc oxide nanoparticles (ZnO NPs)	0.00	0.01125	0.02250
[*]Nutrient specifications of TMR (g/kg dry matter, DM)			
Dry matter (DM), g/kg	1000.00		
Metabolizable energy, Mcal/kg DM	1.90		
Crude protein (CP), g/kg DM	146.50		
Crude fat, g/kg DM	10.80		
Ash, g/kg DM	114.20		
Neutral detergent fibre (NDF), g/kg DM	524.60		
Acid detergent fibre (ADF), g/kg DM	315.50		
Acid detergent lignin (ADL), g/kg DM	87.20		
Non-fibrous carbohydrate (NFC), g/kg DM	203.90		
Zinc (Zn), mg/kg DM	76.44		

[a] Control = basal diet (0 mg ZnO NPs/kg DM TMR).
[b] T1 = Control +11.25 mg ZnO NPs/kg DM TMR.
[c] T2 = Control+22.50 mg ZnO NPs/kg DM TMR.
[d] CP; crude protein.
[l] Each 3 kg of vitamin and mineral mixture contains vit. A 1200000 IU, vit. D_3 200000 IU, vit. E 10000 mg, vit. K_3 2000 mg, vit. B_1 2000 mg, vit. B_2 2000 mg, vit. B_6 2000 mg, manganese oxide 600000 mg, ferrous sulfate 30000 mg, copper oxide 10000 mg, potassium iodide 1000 mg, sodium selenite 100 mg, cobalt carbonate 100 mg, carrier ($CaCO_3$) up to 3 kg (AGRI-VET Corp. 10th of Ramadan City A2, Egypt).
[*] All nutrient specifications of TMR (g/kg DM) were analyzed for the basal diet (control) while Metabolizable energy (Mcal/ kg DM TMR) was calculated from NRC (2001).

in the oven-dried feed samples was determined by an atomic absorption spectrometer (SavantAA Σ AAS, GBC Scientific Equipment, 2–4 Lakewood Boulevard 1135, Braeside Victoria 3195, Australia).

The individual feed intake and refusal of goats was recorded daily. Body weight of goats was recorded at the beginning of the experiment (1st week post-kidding), weekly, and then at the end of the experimental period (12th week post-kidding). Feed conversion ratio was calculated dividing the feed intake by the weight gain.

2.3. Ultrasound examination and measurements of meat yield

Ultrasound examination for all experimental goats was performed by portable ultrasound (ECO-3 EXPERT, CHISON, China) using a 7 L V 7.5 MHz B-mode probe. Ultrasound scanning of *L. dorsi* muscle depth and area as well as the back fat at two measuring points (the 1 st measuring point; P1; between the 12th- and 13th-rib intercostal spaces and the 2nd measuring point; P2; between the 3rd- and 4th-intervertebral spaces of the lumbar vertebrae) at the thoracic and lumbar regions, respectively according to Agamy et al. (2015) and Sahin et al. (2008) with some modifications. Briefly, the goats were manually immobilized, hair was shaved at P1 and P2, and then the acoustic gel was applied to give a good contact between the probe and the skin of the animals. At P1, the transducer was placed on the animal's left side about 4 cm lateral to the vertebral column. The pressure on the transducer head was kept to a minimum to avoid compression of the measured fat and muscle. Ultrasonic images of muscle shadowing (MS) and rib-related acoustic shadowing (RS) at P1 were taken in duplicate to ensure operator repeatability and the thickest point was considered. After a scanned image was captured, the following ultrasound measurements were recorded; the fat thickness covering *L. dorsi* muscle (UFT; mm), the eye muscle depth (ULMD; mm), the eye muscle area (ULMA; cm2), and the eye muscle perimeter (ULMP; cm) using an electronic caliper of the scanner. At P2, the transducer was placed to capture scanned ultrasound images of muscle shadowing (MS) and transverse process-related acoustic shadowing (TS) in the same manner as previously described at P1 while measuring the same ultrasound measurements.

2.4. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using the general linear model (GLM), Univariate of IBM SPSS statistics 20. The individual goat was used as an experimental unit. Tukey's post hoc test (Tukey, 1953) was used for multiple comparisons among the three dietary groups with a confidence level at $p \leq 0.05$.

3. Results and discussion

The basal diet offered to the goats in the current study contains an adequate Zn (76.44 mg/kg DM TMR) as shown in Table 1 which had maintained their performance (data not shown). Our finding was consistent with the previously analyzed basal diet (73.306 ppm Zn) which was adequate for the growth and performance of goats (Aditia et al., 2014).

Dietary supplementation of ZnO NPs at the studied levels revealed no effect on all growth performance parameters of Shami goats, including average daily feed intake, average daily weight gain, and feed conversion compared to control ($p > 0.05$) (Table 2). The insignificant effect of ZnO NPs supplementation on performance of Shami goats was consistent with previous reports in ruminants, which found that dietary Zn concentrations had no effect on growth performance, including daily gain and feed efficiency, of beef steers (Malcolm-Callis et al., 2000).

The effect of ZnO NPs supplementation on the meat yield of *L. dorsi* muscle at both thoracic (P1) and lumbar (P2) regions is illustrated in Table 3 and Fig. 1. Dietary supplementation of ZnO NPs at 11.25 and 22.50 mg/kg DM TMR, respectively, had significantly increased fat thickness (UFT; mm), muscle depth (ULMD; mm), eye muscle area (ULMA; cm2), and eye muscle perimeter (ULMP; cm) of *L. dorsi* muscle located at the thoracic (P1) and lumbar (P2) regions (Table 3) ($P < 0.05$). Ultrasonographic examination of *L. dorsi* muscle at both thoracic and lumbar regions of Shami goats revealed a substantially higher density of muscle shadowing (MS) and rib related acoustic shadowing (RS) in T1 and T2, respectively, in comparison with the control diet (Fig. 1, A–C and D–F).

The meat yield and ultrasound evaluation findings of the *longissimus dorsi* muscle, including depth, area, and perimeter, were also found to be positively related to the dietary level of ZnO NPs supplemented, indicating a dose-dependent increase. The current study findings are consistent with previous findings in crossbred beef steers where Zn supplementation improved meat quality, yield grade, marbling, and back fat (Spears and Kegley, 2002). Also, a quadratic increase in subcutaneous fat thickness and yield grade was observed when zinc was supplemented to the diets of steers (Malcolm-Callis et al., 2000). In the present study, the improved meat yield of eye muscle might be attributed not only to the protective antioxidant mechanisms of Zn on lipid peroxidation but also to the lipid-modulating effect of the Zn. The results in the current study were supported by previous reports in which Zn acts

Table 2
The effect of ZnO NPs supplementation on growth performance of Shami goats.

	[1]Diets			[2]SEM	[3]P value
	Control	T1	T2		
Body weight (kg):					
1st week post-kidding	38.50	38.40	38.40	3.37	1.00
12th week post-kidding	49.80	50.90	51.80	2.79	0.89
Average daily gain (g/d)	134.60	148.80	159.50	12.37	0.38
Average daily feed intake (kg/d)	1.96	2.00	2.05	0.05	0.43
Average feed conversion ratio (kg/kg)	15.56	14.78	14.14	1.80	0.86

[1] Diets: Control = basal diet (0 mg ZnO NPs/kg DM TMR), T1 = Control diet +11.25 mg ZnO NPs/kg DM TMR, T2 = Control diet +22.50 mg ZnO NPs/kg DM TMR.
[2] SEM, standard error of the mean.
[3] All data are expressed as the mean ± SEM. Means without common superscripts ($^{a-c}$) in a raw are significantly diffent ($p \leq 0.05$).

Table 3
The effect of ZnO NPs supplementation on the meat quality of *L. dorsi* muscle at the thoracic and lumbar regions of Shami goats.

	[1]Diets			[2]SEM	[3]p value
	Control	T1	T2		
Ultrasound measurements of *L. dorsi* muscle between the 12th- and 13th-rib intercostal spaces					
Fat thickness (UFT; mm)[2]	0.78^c	1.16^b	1.92^a	0.06	<0.001
Muscle depth (ULMD; mm)[2]	0.48^c	1.07^b	1.81^a	0.05	<0.001
Eye muscle area (ULMA; cm^2)[2]	3.29^c	4.60^b	6.07^a	0.14	<0.001
Eye muscle perimeter (ULMP; cm)[2]	5.00^c	9.80^b	22.20^a	1.35	<0.001
Ultrasound measurements of *L. dorsi* muscle between the 3rd- and 4th-intervertebral spaces of the lumbar vertebrae					
Fat thickness (UFT; mm)	0.97^c	1.57^b	2.56^a	0.09	<0.001
Muscle depth (ULMD; mm)	0.65^c	0.79^b	1.05^a	0.04	<0.001
Eye muscle area (ULMA; cm^2)	1.23^c	1.51^b	1.87^a	0.05	<0.001
Eye muscle perimeter (ULMP; cm)	5.00^c	9.00^b	23.40^a	1.24	<0.001

[1] Diets: Control = basal diet (0 mg ZnO NPs/kg DM TMR), T1 = Control diet +11.25 mg ZnO NPs/kg DM TMR, T2 = Control diet +22.50 mg ZnO NPs/kg DM TMR.
[2] SEM, standard error of the mean; UFT, the fat thickness covering *L. dorsi* muscle; ULMD, the eye muscle depth; ULMA, the eye muscle area; ULMP, the eye muscle perimeter.
[3] All data are expressed as the mean± SEM; Means without common superscripts ($^{a-c}$) in a raw are significantly diffent ($p \leq 0.05$).

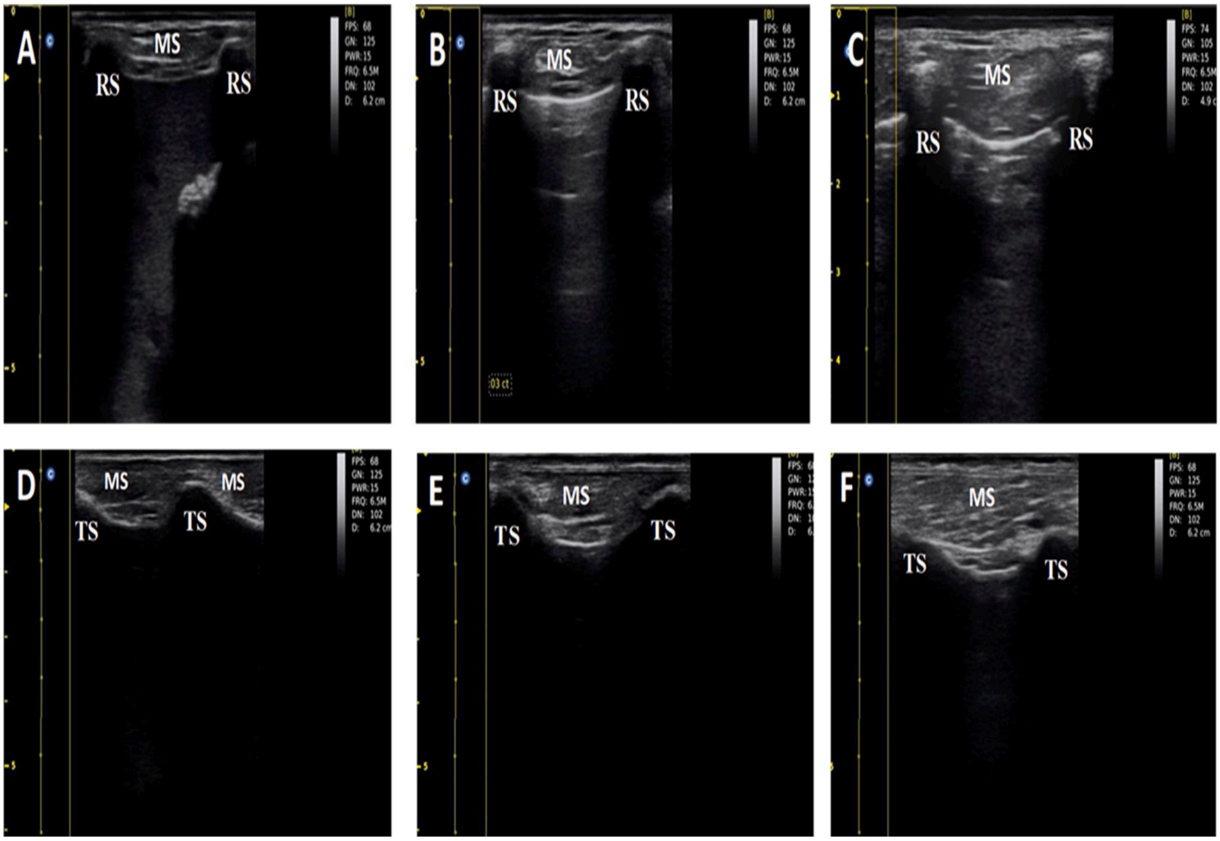

Fig. 1. Ultrasound scanning of *L. dorsi* muscle between the 12th- and 13th-rib intercostal spaces (A–C) of Shami goats fed Control, T1, and T2 diets, respectively. Ultrasound scanning of *L. dorsi* muscle at 3rd- and 4th-intervertebral spaces of the lumbar vertebrae (D–F) in Shami goats fed Control, T1, and T2 diets, respectively. Control = basal diet (0 mg ZnO NPs/kg DM TMR); T1 = Control +11.25 mg ZnO NPs/kg DM TMR; T2 = Control +22.50 mg ZnO NPs/kg DM TMR. MS, Muscle Shadowing; RS, Rib-related acoustic Shadowing; and TS, transverse process-related acoustic shadowing.

as a powerful antioxidant to protect cell membranes (Jiang et al., 2018) and prevents lipid peroxidation (Sloup et al., 2017). The lipid-modulating effect of Zn was previously studied in broilers in which Zn supplementation increased the intramuscular fat content of the breast muscle (Liu et al., 2011) and was confirmed by the higher expression level of the fat-metabolism related genes in broilers fed the diet supplemented with Zn (Liu et al., 2015). The current ultrasound scanning results, as well as previous studies (Grill et al., 2015), affirm the practicability of ultrasound method for assessing meat yield in live farm animals.

4. Conclusions

Dietary ZnO NPs supplementation, 11.25 and 22.50 mg/kg DM, improved meat yield in Shami goats compared to control animals. The ultrasound assessment of the thoracic and lumbar *L. dorsi* regions of ZnO NP-supplemented Shami goats revealed a dose-dependent increase in muscular density, fat thickness, muscle depth, perimeter, and area. Future biochemical, genetic, and meat quality studies would aid in explaining the mechanism underlying the positive effect of low dietary ZnO NPs doses on meat yield.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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