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Effect of Zinc Oxide and Selenium Nanoparticles on Milk Production Efficiency and Related Gene Expression in Egyptian Baladi Goats

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Abstract

This study assessed the impact of oral administration of zinc oxide (ZnO) and selenium (Se) nanoparticles on milk production and gene expression in Baladi goats. Twenty-two pregnant females, 30 days before parturition, were divided into five groups: Group 1 (n=4) served as control; Groups 2 and 3 (n=4 each) received 150 mg ZnO and 0.3 mg of Se in microparticle (MP) forms, respectively; Groups 4 and 5 (n=5 each) received 15 mg ZnO and 0.03 mg Se in nanoparticle (NP) forms, respectively. Milk yield, composition, and gene expression related to milk production were analyzed. Results indicated a significant increase ($P<0.001$) in dry matter intake in the ZnO-NPs and Se-NPs groups, with an improved feed conversion ratio. Daily and overall milk yield, as well as fat-corrected milk yield, were highest in the NPs groups, particularly ZnO-NPs. Milk composition remained unchanged. Gene expression analysis showed significant upregulation of POU1F1, IGF-1, PPARγ, CSN2, and FASN in the NPs groups compared to the MPs and control groups, with the highest expression levels observed in the Se-NPs group. These findings suggest that ZnO-NPs and Se-NPs enhance milk production efficiency and upregulate key genes involved in milk yield in Baladi goats.

Keywords: Zinc oxide nanoparticles; Selenium nanoparticles; Milk yield; Milk composition; Gene expression; Baladi goats; RT-PCR

1. Introduction

The demand for increased animal production is critical to address the challenges posed by global overpopulation, with the world population projected to reach 10 billion by 2050 [1]. Goats play a significant role in dairy production due to the high quality and nutritional value of their milk, which benefits both children and the elderly, as well as those with food allergies [2-4]. Recently, the demand for goat milk has been rising, particularly in Mediterranean countries, including Egypt, where goat milk is valued for its use in producing a variety of cheeses with distinct textures, flavors, and colors [5-7]. Consequently, improving goat breeding practices is essential for enhancing milk production. While extensive research has been conducted on improving milk production in cattle using molecular approaches [8-10], similar advancements for goats have been limited. The economic importance of goat milk production has driven research into identifying genomic regions that affect dairy traits.

Several key genes have been identified as crucial for milk yield and quality in farm animals, including goats. These genes include POU1F1 (POU domain class 1 transcription factor 1), IGF-1 (insulin-like growth factor 1), PPARγ (peroxisome proliferator activated receptor gamma), CSN2 (β-casein), and FASN (fatty acid synthase) [4, 9,11,12].

The POU1F1, also known as Pituitary-specific transcription factor 1, is a major regulator of prolactin, thyroid-stimulating hormone (TSH), and growth hormone, all of which are essential for mammary gland development and milk production [11-13].

IGF-1 plays a crucial role in cell proliferation and growth, affecting various metabolic pathways. It stimulates myogenesis, glucose absorption, lipid synthesis, and influences cell cycle activation, progesterone production, and protein synthesis [14-16]. The expression of IGF-1 plays a crucial role in enhancing mammary gland activity by increasing arterial blood flow, thereby positively affecting both milk yield and quality [17].

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PPARγ is a key regulator of adipocyte differentiation and transcriptional activity, significantly influencing milk fat synthesis. Higher levels of PPARγ expression are associated with lower fat content in milk, whereas reduced expression results in increased fat levels [9, 12]. In goats, PPARγ expression controls milk fat secretion from the mammary gland [18, 12].

The CSN2 gene encodes β-casein, a major protein fraction in goat milk that significantly influences milk yield [12]. Elevated CSN2 expression is linked to enhanced milk yield and higher milk protein content in goats [19, 20, 12].

The FASN gene is highly expressed in goat colostrum and is essential for maintaining lactation [21, 4]. It plays a critical role in synthesizing all types of fatty acids, including short, medium, and long chain; therefore, it is a key gene for distinguishing high-quality animal products based on their fatty acid profiles. Inhibition of FASN reduces fatty acid synthesis, particularly medium-chain fatty acids, in the mammary gland [22, 4].

Environmental factors, especially nutrition, significantly impact animal productivity and gene expression. Studies have shown that nanoparticle supplementation in animal diets can enhance production and improve performance [23, 24]. Zinc oxide and selenium nanoparticles (ZnO-NPs and Se-NPs) have demonstrated positive effects in enhancing meat production in animals like pigs [25] and rabbits [26]. To date, there is limited literature on the impact of ZnO-NPs and Se-NPs on milk production and on the expression of key genes related to milk production. However, their effects on gene expression have been studied in other contexts in mice [27], rats [28], and goats [29].

Milk from native Baladi goats is known for its nutritional value, benefiting both children and the elderly, and is important in the production of high-quality cheeses. However, research on enhancing its properties through nanotechnology and molecular genetics is scarce. This study aims to investigate the effects of zinc oxide and selenium nanoparticles on milk production and the expression of key genes (POU1F1, IGF-1, PPARγ, CSN2, and FASN) in native Egyptian Baladi goats.

2. Experimental

2.1. Preparation of nanoparticles

ZnO-NPs were synthesized by refluxing 3.942 g of zinc acetate (Sigma Aldrich, Germany) in 1 L of ethanol containing 1.44 g of NaOH (El-Gomhouria, Egypt) for 2 hours at 70°C. Following reflux, deionized water was added, and the mixture was centrifuged at 7000 rpm for 10 minutes. The resulting fine white powder was then calcined at 500°C for 2 hours to obtain ZnO-NPs [30].

Se-NPS were prepared by dissolving 30 mg of sodium selenate ($Na_2SeO_4 \cdot 10H_2O$) (Sigma Aldrich, Germany) in 90 ml of double-distilled water. To this solution, 10 ml of ascorbic acid solution (56.7 mM) (Sigma Aldrich, Germany) was added dropwise while stirring vigorously. Polysorbate (El-Gomhouria, Egypt) was added in a ratio of 0.01 ml to 2 ml of ascorbic acid. The formation of Se-NPs was indicated by a color change in the solution from white to red [31].

2.2. Animals and experimental design

Twenty-two pregnant Baladi goats were utilized in this study, conducted over a period of four months, beginning 30 days prior to their expected parturition. The goats were housed at the Animal Production Research Institute, Agricultural Research Centre, located in Sids City, Beni-Suef Governorate, Egypt. The goats were randomly assigned to five groups. The number of animals used in this study was determined based on their availability at the farm and adherence to ethical guidelines for animal research. All treatments were dissolved in 50 ml of drinking water. Group 1 (n=4) served as the control and received drinking water without additives. Groups 2 and 3 (n=4 each) received 150 mg ZnO-MPs and 0.3 mg Se-MPs, respectively. Groups 4 and 5 (n=5 each) received 15 mg ZnO-NPs and 0.03 mg Se-NPs, respectively. Treatments continued until the goats' kids were weaned at 90 days post-parturition, covering the productive and reproductive cycles, including parturition and lactation. The feed conversion ratio (FCR) was calculated as total feed intake divided by average daily milk yield to assess overall performance. For milk sampling, kids were separated from their mothers 24 hours before milking. The does were then milked until the udder was fully emptied, and approximately 100 grams of milk per doe were collected and stored at –20°C until analysis. Sodium azide was added to the milk samples at a concentration of 0.1% (w/v) to preserve them. Fats, proteins, lactose, and solids non-fat (SNF) were analyzed using a milk content automatic analyzer (LactoStar, Funke Gerber, Berlin, Germany) at the Dairy Production Laboratory, Dairy Science Department, National Research Centre, Giza, Egypt. Fat-corrected milk (FCM) [32] and energy-corrected milk (ECM) [33] were calculated as follows:

(1) 4% FCM = 0.4 (milk yield, g) + 15 (fat yield, g)

(2) ECM= 0.327 (Milk yield, kg) + 12.95 (Fat yield, kg) + 7.20 (Protein, kg)

2.3. Gene expression analysis

2.3.1. RNA extraction

Milk samples (25-50 mL) were collected from each experimental group and centrifuged at 2000 xg for 15 minutes at 4°C. The supernatant was discarded, leaving 1-2 mL of liquid in the tube, which was then mixed with an equal volume of phosphate-buffered saline (PBS, pH 7.2) at 4°C. After a second centrifugation, PBS containing 0.5 mM EDTA was added to

the pellets, and a final centrifugation was performed at the same speed. The pellets were homogenized in liquid nitrogen with 1 mL of TRIzol® Reagent for RNA extraction. The homogenized samples were incubated at room temperature for 15 minutes, then mixed with 0.2 mL of chloroform per 1 mL of TRIzol®. Following vigorous vortexing and a 3-minute incubation, the samples were centrifuged at 12,000 xg for 15 minutes at 4°C. The mixture separated into three layers. The clear aqueous upper phase, which contained the RNA, was transferred to a new tube. RNA was then precipitated by adding 0.5 mL of isopropanol per 1 mL of TRIzol®. The samples were incubated at 15-30°C for 10 minutes, centrifuged at 12,000 xg for 10 minutes at 4°C, and the RNA pellet was washed with 1 mL of 75% ethanol, vortexed, and centrifuged at 7,500 xg for 5 minutes at 4°C. After air-drying the pellet for 10 minutes, RNA was dissolved Diethyl pyrocarbonate (DEPC)-treated water [34]. Total RNA was treated with 1 U of RQ1 RNase-free DNase (Invitrogen, Germany) to remove DNA residues. RNA purity was checked using the 260/280 nm ratio (1.8-2.1), and integrity was assessed by formaldehyde-containing agarose gel electrophoresis, with RNA visualized using ethidium bromide staining to confirm the presence of 28S and 18S ribosomal RNA bands. RNA aliquots were used immediately or stored at -80°C [12, 35].

2.3.2. Reverse transcription (RT) reaction

The RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Germany) was used to synthesize cDNA from total RNA isolated from milk samples. A total of 5 µg of RNA was reverse transcribed in a 20 µL reaction volume, containing 50 mM $MgCl_2$, 5x RT buffer, 10 mM of each dNTP, 50 µM oligo-dT primer, 20 U ribonuclease inhibitor, and 50 U M-MuLV reverse transcriptase [36]. The RT reaction was carried out in a thermocycler (Biometra GmbH, Göttingen, Germany) at 25°C for 10 minutes, followed by 42°C for 1 hour, and then terminated by heating at 99°C for 5 minutes. The resulting cDNA was flash cooled on ice and used for Real-Time PCR (RT-PCR).

2.3.3. Real time-polymerase chain reaction

The StepOne™ Real-Time PCR System from Applied Biosystems (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine gene expression. RT-PCR reactions were set up in 25 µL mixtures containing 1× SYBR® Premix Ex Taq™ (TaKaRa, Biotech. Co. Ltd.), 0.2 µM sense primer, 0.2 µM antisense primer, and 5 µL of cDNA template). [37, 38].
The RT-PCR program consisted of three main stages. The first step was an initial denaturation at 95.0°C for 3 minutes. The second step included 40 cycles, each with three phases: denaturation at 95.0°C for 15 seconds; annealing at 55.0°C for 30 seconds; and extension at 72.0°C for 30 seconds. The third stage involved a melting curve analysis with 71 cycles starting at 60.0°C, increasing by 0.5°C every 10 seconds, up to 95.0°C. The expression levels of target genes were normalized to the housekeeping gene Ribosomal Protein Lateral Stalk Subunit P0 (RPLP0) using the $2^{-\Delta\Delta Ct}$ method. [39, 40]. Gene-specific primers, used for this study, are listed in Table 1.

Table 1: Primer sequences and amplicon sizes for milk production-related genes

Genes	Accession Number	Primer Sequences 5′- 3′	Size (bp)
POU1F1	NM_001285673.1	F: CTG GAG AGA CAC TTT GGA GAA C R: CCA AAC CCT CAC CAC TTC TT	99
IGF-1	NM_001285697.1	F: TCC TCC TCG CAT CTC TTC TAT R: GAG AGC ATC CAC CAA CTC AG	105
PPARγ	NM_001285658.1	F: GTT CAA CGC GCT GGA ATT AG R: GGG CTT CAC ATT CAG CAA AC	97
CSN_2	XM_013964699.2	F: TCC TTC ACT TCT TCT CCT CTA CT R: TTG AGT TCT TCC TGC TCT CTT	111
FASN	NM_001285629.1	F: GCACACAATATGGACCCCCA R: CATGCTGTAGCCTACGAGGG	183
RPLP0	NM_001314331.1	F: CAA CCC TGA AGT GCT TGA CAT R: AGG CAG ATG GAT CAG CCA	227

F: Forward primer, R: Reverse primer

2.4. Statistical analysis

The Data were analyzed using one-way analysis of variance (ANOVA) following the general linear model approach outlined by Steel and Torrie (1980) [41], utilizing the SAS software [42]. Differences among significant means were identified using Tukey's Honestly Significant Difference (HSD) test.

3. Results

3.1. Characterization of ZnO and Se nanoparticles

Figure 1A and B present the TEM images of ZnO-NPs and Se-NPs, respectively, alongside their corresponding XRD patterns. The ZnO-NPs displayed a particle size of less than 100 nm (Figure 1A). XRD analysis confirmed the ZnO-NPs crystalline structure, with prominent peaks at 2θ angles of 32°, 34.4°, 36.4°, 47.7°, and 56.7°. Similarly, the Se-NPs (Figure 1B) exhibited particle sizes below 50 nm. The XRD data for Se-NPs revealed peaks at 2θ angles of 23.9°, 30.0°, 45.7°, 52.0°, 55.9°, and 65.5°.

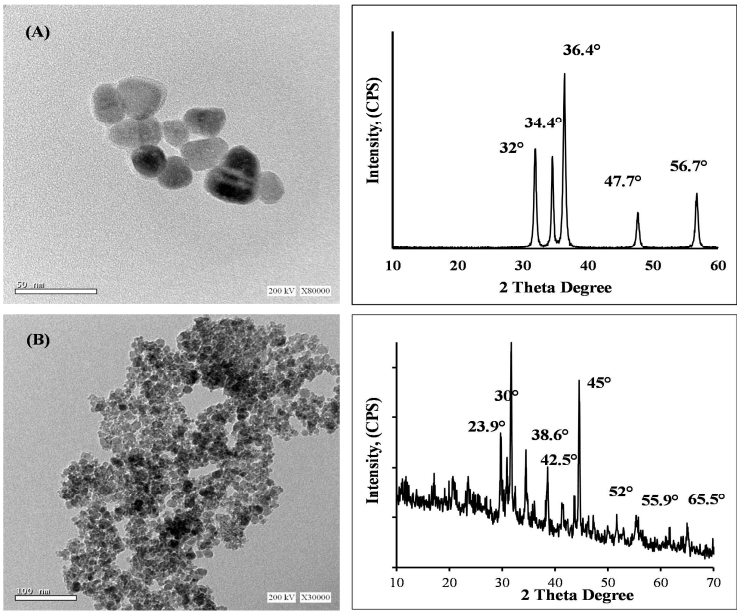

Figure 1: The TEM image of (A) ZnO-NPs and (B) Se-NPs and their corresponding XRD pattern.

3.2. Milk yield and composition

The results in Table 2 showed significant differences among treatment groups ($P < 0.001$) compared to the control. Goats treated with NPs had the highest total dry matter intake (TDMI) and the lowest FCR, with no significant differences between ZnO and Se. Similarly, no significant differences in TDMI or FCR were observed between goats receiving the MP forms of ZnO and Se. These findings suggest that NP forms of both ZnO and Se were more effective in improving FCR, contributing to enhanced milk production. FCR, calculated as grams of TDMI consumed per gram of milk yield, was significantly ($P < 0.001$) improved with both forms of ZnO and Se supplementation compared to the control group.

The results in Table 2 also revealed that supplementation with nano-form of zinc and selenium had a significant positive effect on both daily milk yield and overall milk production during the lactation period. Total milk production was 41.54% higher in the group receiving ZnO-NPs and 25.37% higher in the group receiving Se-NPs compared to the control group. The ZnO-NP group exhibited the highest milk production.

Moreover, neither zinc nor selenium, in either micro or nano form, significantly impacted milk components such as fat, protein, lactose, non-fat solids, total solids, and ash. However, the group supplemented with ZnO-NPs showed the highest fat-corrected milk yield (FCM, g/d) and energy-corrected milk yield (ECM, g/d), followed by the Se-NPs group ($P < 0.05$). The ZnO-NPs group also consistently had higher yields of fat, protein, SNF, total solids, and ash compared to all other groups ($P < 0.05$). These results indicate that ZnO-NPs supplementation can effectively improve the nutritional quality of milk, making it a valuable addition to dairy production.

Table 2: Effect of Nanoparticles on Feed efficiency, milk production and milk composition

Items	Experimental groups					±SE	*P* value
	Control	ZnO-MPs	Se-MPs	ZnO-NPs	Se-NPs		
Feed efficiency							
TDMI (g)	1343.00^{bc}	1295.00^{c}	1319.25^{c}	1448.80^{a}	1400.00^{ab}	20.11	0.0002
FCR	2.34^{a}	2.03^{bc}	2.16^{b}	1.78^{d}	1.94^{c}	0.51	<0.0001
Milk production							
Milk yield, g/d	575.13^{a}	638.58^{a}	613.96^{a}	814.00^{c}	720.93^{b}	19.97	<0.001
Total Milk yield, kg	51.76^{a}	57.48^{a}	55.26^{a}	73.26^{c}	64.89^{b}	1.80	<0.001
Milk composition							
Fat, %	3.42	3.58	3.57	3.74	3.74	0.08	0.754
Protein, %	3.31	3.38	3.84	3.52	3.20	0.08	0.101
Lactose, %	4.61	4.68	4.56	4.69	4.70	0.05	0.889
SNF, %	8.94	9.36	9.45	9.22	8.91	0.14	0.683
Total Solids, %	12.36	12.94	13.02	12.96	12.66	0.17	0.783
Ash, %	1.02	1.00	1.06	1.01	1.02	0.02	0.803
FCM, g/d	524.98^{a}	598.03^{a}	570.36^{a}	782.34^{c}	692.85^{b}	21.90	<0.001
ECM, g/d	579.69^{a}	659.66^{ab}	650.44^{a}	867.24^{c}	750.60^{ab}	23.64	<0.001
Milk composition yield							
Fat yield, g/d	19.66^{a}	22.84^{ab}	21.65^{ab}	30.45^{c}	26.97^{bc}	0.99	<0.001
Protein yield, g/d	19.03^{a}	21.54^{a}	23.51^{a}	28.72^{c}	23.01^{a}	0.85	<0.001
Lactose yield, g/d	26.48^{a}	29.88^{a}	27.93^{a}	38.19^{c}	33.82^{b}	1.00	<0.001
SNF yield, g/d	51.36^{a}	59.60^{ab}	57.96^{ab}	75.09^{c}	64.18^{b}	1.95	<0.001
Total Solids yield, g/d	71.02^{a}	82.45^{bc}	79.61^{ab}	105.54^{d}	91.15^{c}	2.81	<0.001

a, b, c and d, means with different superscripts, within each row are significantly different at $P < 0.05$.
Feed conversion ratio (FCR) = total dry matter intake (TDMI) (g) / Avg. daily milk yield (g).
Fat-corrected milk (FCM)= 0.4 (milk yield, g) + 15 (fat yield, g)
Energy-corrected milk (ECM)= 0.327 (Milk yield, kg) + 12.95 (Fat yield, kg) + 7.20 (Protein, kg)

3.3. Milk production-related gene expression

This study investigated the expression levels of key genes related to milk production, including POU1F1, IGF-1, PPARγ, CSN2, and FASN, in milk samples from goats treated with either MPs or NPs of ZnO and Se. Figure 2 illustrates the gene expression changes.

The expression levels of these genes significantly increased in the treated groups compared to the control group. In animals treated with MPs of Zn or Se, the expression levels of POU1F1, IGF-1, PPARγ, CSN2, and FASN were significantly higher ($P < 0.05$) than in the control group. Although animals treated with Se-MPs exhibited higher gene expression than those treated with Zn-MPs, the difference was not statistically significant. Although animals treated with Se-MPs exhibited higher gene expression than those treated with Zn-MPs, the difference was not statistically significant.

In contrast, animals treated with Se-NPs demonstrated a significant increase ($P < 0.01$) in the expression of the target genes compared to the control group and those treated with Zn-MPs and Se-MPs. Additionally, the Se-NP-treated group showed significantly higher expression levels compared to the ZnO-NPs group ($P < 0.05$). However, the expression levels of PPARγ were relatively similar in the groups treated with ZnO-NPs and Se-NPs, without significant differences.

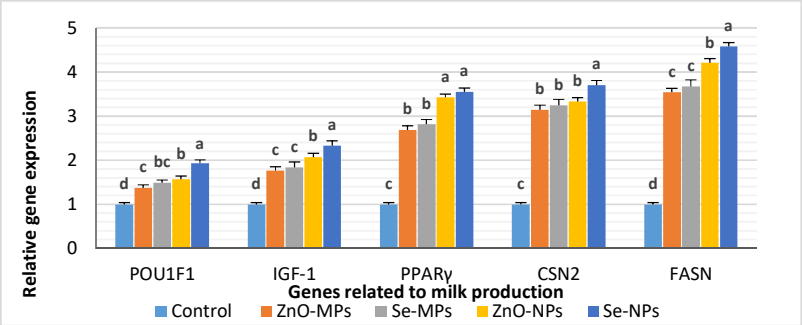

Figure 2: Expression levels of POU1F1, IGF-1, PPARγ, CSN2, and FASN genes in milk samples from goat groups treated with MPs and NPs of ZnO and Se. Data are presented as mean ± SEM. Within each gene, treatment groups labelled with different superscript letters (a, b, c, d) are significantly different ($P < 0.05$).

4. Discussion

4.1. Characterization of ZnO and Se nanoparticles

The characterization of ZnO and Se nanoparticles demonstrated that ZnO-NPs had a particle size of less than 100 nm, while Se-NPs exhibited even smaller sizes below 50 nm. The distinct crystalline structures of both nanoparticles were confirmed through XRD analysis. The prominent peaks in the XRD patterns of ZnO-NPs correspond to specific crystal planes, indicating their crystalline nature. Specifically, the observed peaks at 32° (100), 34.4° (002), 36.4° (101), 47.7° (102), and 56.7° (110) reflect the typical structure of ZnO [43].

Similarly, the XRD data for Se-NPs revealed reflections at 23.9° (100), 30.0° (101), 45.7° (111), 52.0° (201), 55.9° (003), and 65.5° (210), characteristic of the pure hexagonal phase of selenium crystals [44]. These findings suggest that both types of nanoparticles possess structural properties that could influence their bioactivity and effectiveness in applications.

4.2. Milk yield and composition

The present results (Table 2) revealed that goats treated with NPs exhibited higher total TDMI, lower FCR, and significantly higher milk yield compared to both the MPs and control groups. These results align with earlier research suggesting that increased dry matter and crude protein degradability may contribute to improved performance [45]. Notably, FCR, calculated as grams of TDMI per gram of milk yield, significantly ($P< 0.001$) improved with both nano and micro forms of ZnO and Se supplementation compared to the control group, consistent with previous findings by Vignola et al. [46] and Xun et al. [47].

Zhao et al. [48] suggested that the enhanced effects observed with ZnO-NPs supplementation may be attributed to the unique shape and small size of NPs, which allow for efficient cellular penetration. Improved TDMI and FCR following ZnO supplementation have also been supported by studies of Garg et al. [49] and Fadayifar et al. [50]. ZnO supplementation enhances nutrient absorption, gut health, immune response, and digestive enzyme activity in livestock, leading to improved feed efficiency and increased milk production. It also helps reduce gut inflammation, contributing to better overall growth performance [51]. Additionally, ZnO-NPs has demonstrated benefits in reducing somatic cell counts (SCC) in cows with subclinical mastitis, leading to improved milk production, udder health and overall farm economics compared to conventional ZnO sources [52]. Supplementation with nano-Zn at doses of 10 and 20 ppm has proven more effective than inorganic Zn in reducing SCC without affecting milk yield or composition [53].

Selenium supplementation has also been linked to improved milk production [54] and enhanced milk fat and protein content [55]. Research has shown that nano-Se increases Se concentration and glutathione peroxidase activity in milk and blood, as well as upregulates mammary gland mRNA expression levels [56]. Additionally, Nano-Se has been shown to promote the proliferation and viability of mammary epithelial cells and enhance milk fatty acid synthesis. These findings suggest that nano-Se has the potential to boost lactation performance and mammary gland development [54, 57].

However, the results of this study contrast with those of Bakhshizadeh et al. [58], who found no significant effects of nano zinc on feed intake, milk production, or composition. Discrepancies in the impact of zinc supplementation on milk yield and composition have been observed across different studies. For example, while Wang et al. [28] and Kellogg et al. [59] reported positive effects of zinc on milk production, others, such as Sobhanirad et al. [60] and Zali et al [61] found no significant impact.

The group treated with ZnO-NPs exhibited the highest FCM and ECM values, followed by the group treated with Se-NPs ($P < 0.05$). ZnO-NPs have been associated with enhanced nutrient digestibility, particularly of acid detergent fiber and cellulose, resulting in enhanced feed intake, nutrient absorption, and increased milk production. Additionally, they raise blood Zn levels in livestock without causing toxicity [62, 63]. Similarly, Se-NPs have been shown to improve Se bioavailability, nutrient digestibility, and increase Se concentration in blood and tissues more effectively than other forms of Se [64-66].

The current study also found that treatment with ZnO-NPs and Se-NPs did not significantly impact milk composition. This observation is consistent with findings of Ianni et al. [67] and Cortinhas et al. [68], who reported no significant effects of ZnO supplementation on feed intake, milk production, or composition Similarly, Cruickshank et al. [69] found no significant changes in daily DMI or milk yield with selenium supplementation, whether organic or inorganic.

4.3. Gene expression

The findings in Figure 2 revealed significant upregulation in the expression levels of POU1F1, IGF-1, PPARγ, CSN2, and FASN genes, all of which are associated with improved milk production, in the NPs groups compared to the MPs and control groups. These upregulations were most pronounced in the Se-NPs groups, indicating that Se-NPs were more effective than ZnO-NPs in enhancing the expression of these candidate genes. To our knowledge, this is the first study to investigate and report on the effects of ZnO-NPs and Se-NPs on gene expression related to milk production in goats.

While our findings are innovative in the realm of milk production, previous studies have documented the impacts of ZnO-NPs and Se-NPs on gene expression in other contexts. For instance, Wang et al. [28] examined alteration in mRNA expression of zinc metabolism-related genes, such as metallothionein, in mice fed diets containing either 50 mg/kg or 500

mg/kg of nano zinc oxide. Their research revealed a significant increase in the expression levels in animals consuming a higher concentration of nanoparticles, suggesting a dose-dependent effect.

In another study, Kim et al. [70] explored the therapeutic effects of an herbal medicine consisting of bamboo salt and ZnO-NPs on inflammation. Their results demonstrated a reduction in mRNA expression levels of inflammatory cytokines, likely through the inhibition of nuclear factor kappa B (NF-κB) activation, highlighting the potential of ZnO-NPs to modulate immune responses.

Goma et al. [29] reported that rats injected intraperitoneally with ZnO-NPs showed enhanced mRNA expression of steroidogenesis-associated genes and anti-apoptotic genes compared to controls, further emphasizing the broad impact of ZnO-NPs on gene regulation. Similarly, Abedin et al. [30] found that cryopreserved buck semen supplemented with ZnO-NPs or Se-NPs exhibited upregulation of heat shock protein (HSP) genes, specifically HSP70 and HSP90, compared to controls, suggesting a protective role of NPs under stress conditions.

In aquatic species, Fasil et al. [71] observed that zebrafish fed a diet supplemented with a combination of ZnO-NPs and Se-NPs showed improved expression of growth-related genes (GH and IGF-1), enhanced growth performance (increased length and weight), and better overall development compared to those fed either ZnO-NPs or Se-NPs alone, or the control diet. These findings demonstrate the potential for nanoparticle supplementation to enhance physiological outcomes across different species and biological processes.

Overall, the present study adds to the growing body of research demonstrating the potential of ZnO-NPs and Se-NPs to modulate gene expression and improve production-related traits in livestock, with promising implications for practical applications in dairy production.

5. Conclusion

This study demonstrates that ZnO-NPs and Se-NPs significantly enhance milk production, feed efficiency, and the expression of key milk-related genes (POU1F1, IGF-1, PPARγ, CSN2, FASN) in Egyptian Baladi goats, with Se-NPs showing the most significant effect. These results suggest that zinc and selenium particularly, in NP forms, are promising tools for improving dairy production efficiency and genetic regulation in goats. Further investigation could explore the long-term impacts of these nanoparticles on milk quality and animal health to fully harness their potential in sustainable dairy production.

6. Ethics Approval

Milk samples were collected from goats under veterinary supervision and in compliance with local and international guidelines for animal care and use. The collection of milk samples was approved by the Animal Production Research Institute, Agricultural Research Centre, Sids City, Beni-Suef Governorate, Egypt, with written consent. All procedures involving animals were approved by the Institutional Animal Care and Use Committee, National Research Centre, Egypt, with Permit Reference Number: 13050410-1.

7. Conflict of Interest

The authors declare that there is no conflict of interest related to this study.

8. Formatting of funding sources

This study was conducted as part of internal project at National Research Centre, No. 13050 410, titled "Use of Gene Markers, Gene Expression, and Feeding on Nanoparticles of Selenium and Zinc Oxide for Improving Meat and Milk Production in Egyptian Goats," with Prof. Dr. Ibrahim M. Farag as the Principal Investigator.

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10. Authors' Contribution

I.M. Farag: Designed the study, wrote the manuscript. **W.K.B. Khalil** and **H. Mansour:** Conducted gene expression analysis experiments. **I.M. Farag, W.K.B. Khalil, H. Mansour, R. Agamy:** Analyzed and curated the gene expression data and contributed to writing the genetic analysis section. AMD: contributed to writing the genetic analysis section. **A.A. Aboamer:** Conducted the milk composition analysis. **S.M. Ali and M.Y. Mohamed:** Managed the experimental diets throughout the experiment. **A.A. Aboamer, S.M. Ali, M.Y. Mohamed:** Analyzed the data on milk yield and quality and contributed to writing the section on milk production. **M.E. Abd El-Aziz:** Prepared and characterized the nanoparticles. All authors read and approved the final manuscript.

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