Animal Health Research Journal

P-ISSN : 2356-7767 On Line-ISSN : 2535-1524
Journal Homepage: https://animalhealth.ahri.gov.eg/ Research Paper

Risk assessment of *Fasciola hepatica* antibodies in milk and their impact on milk production

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Received in 17/4/2025
Received in revised from 13/5/2025
Accepted in 18/6/2025

Keywords:
Fascioliasis,
Serum biochemical profiles,
Oxidative stress profiles, milk composition.

Abstract

Fascioliasis is an endemic parasitic disease affecting both humans and animals, causing significant health issues and substantial economic losses. This study aimed to evaluate the risk associated with the presence of antibodies against *Fasciola hepatica* in milk and to assess its impact on milk production and composition. Samples of feces, milk, and serum were collected from 100 cattle across various farms in Dakahlia Governorate, Egypt, and analyzed for *Fasciola* infection. The results revealed a 27% infection rate. Infected cattle exhibited significantly elevated hepatic enzyme levels, including aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, and glutamate dehydrogenase, as well as increased serum urea and creatinine levels. Immunological profiling indicated a significant increase in serum interleukin (IL)-4, IL-10, and transforming growth factor-beta levels, alongside a marked decrease in interferon-γ. Oxidative stress markers showed significantly reduced glutathione levels, with elevated serum nitric oxide and malondialdehyde concentrations. Milk analysis demonstrated a significant reduction in fat, solids-not-fat (SNF), protein, and lactose content, coupled with an increase in salt concentration and pH. Additionally, total mesophilic bacterial counts and somatic cell counts in milk were significantly elevated. These findings suggest that *Fasciola hepatica* infection induces oxidative stress, adversely affecting milk composition and quality, thereby leading to considerable economic losses and posing health risks to both animals and humans.

Introduction

Fascioliasis is a globally distributed disease affecting at least 50 countries and imposing substantial economic burdens on production systems **(Fanke *et al*., 2017).** It affects a wide range of animal species, including horses, cat-

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tle, sheep, camels, and others, with reported prevalence rates reaching up to 59% (**Charlier *et al*., 2016).** In Egypt, fascioliasis is an enzootic disease among farm animals, constituting a significant public health concern **(Boray, 1969).** The disease results in approximately 30% losses in milk and meat production, leading to considerable financial detriment, as noted by the Egyptian General Organization of Veterinary Services **(GOVS, 1998).** Fascioliasis is caused by the digenean trematodes *Fasciola hepatica* and *Fasciola gigantica* **(Arjona *et al*., 1995).** Freshwater snails of the genus *Lymnaea truncatula* serve as intermediate hosts where asexual reproduction occurs, producing numerous metacercariae **(Vignoles *et al*., 2015).** These metacercariae contaminate water sources and, upon ingestion by definitive hosts, penetrate the intestinal wall and migrate to the liver within approximately six weeks. The flukes invade the bile ducts, where they mature and begin egg production **(Andrews, 1999).** This migration through liver parenchyma causes cellular membrane damage and necrosis to hepatocytes and biliary epithelium, leading to elevated serum hepatic enzymes due to leakage **(Behm and Sangster, 2008).** Fascioliasis may also suppress immune responses, increasing susceptibility to various pathogens, particularly bacterial infections.

The impact of fascioliasis and its treatment on milk yield and composition in dairy cows has been previously evaluated **(Novobilský and Höglund, 2009).** For instance, **Vercruysse and Claerebout (2001)** reported reductions in annual herd milk fat and protein content correlated with increased *F. hepatica* antibody levels by 0.06% to 0.09% and 0.05%, respectively. Given these effects, the present study was designed to investigate the impact of Fasciola infection on animal health and its consequent effects on milk constituents.

Materials and Methods

Collection of Samples

A total of 100 dairy cows, aged between 1 and 1.5 years, were selected from farms in Dakahlia Governorate. All animals underwent a thorough clinical examination for fascioliasis. The cows were monitored on the farms for one month while being fed dry chow to observe the presence of Fasciola eggs in their feces.

Rectal fecal samples were collected directly using disposable plastic gloves and labeled accordingly. Individual milk and blood samples were obtained from each cow. Before milk sampling, the udder was thoroughly cleaned, and the teats were disinfected by dipping. Approximately 250 ml of milk was collected per cow, discarding the initial streams to avoid contamination. Milk samples were collected in sterile containers and immediately transported to the laboratory in insulated iceboxes. They were analyzed for compositional parameters and subjected to total mesophilic bacterial count and somatic cell count.

Blood samples (5 ml) were drawn from the jugular vein into sterile tubes. Serum was separated by centrifugation and transferred into labeled sterile Eppendorf tubes for further analysis. Both milk and serum samples were stored at -20°C until further examination. Prior to analysis, frozen milk samples were thawed at room temperature and centrifuged at 3000 rpm for 10 minutes to separate components, as described by **Attia *et al*. (2017).**

The study protocol was approved by the Animal Health Research Institute (AHRI), Agriculture Research Centre (ARC), Giza, Egypt (Approval code:ARC/AHRI/53/25).

Fecal examination

Fecal samples were examined for the presence of Fasciola eggs using the sedimentation technique, considered the gold standard diagnostic method **(Soulsby, 1968).** Briefly, 10 g of feces was thoroughly mixed with 200 ml of water and passed through a sieve to remove large debris. The suspension was then allowed to stand undisturbed for approximately 30 minutes to permit sedimentation of eggs. After decanting the supernatant carefully, the sediment was collected and examined microscopically under a dissecting microscope for the presence of Fasciola eggs.

Enzyme-linked Immunosorbent Assay (ELISA)

Serodiagnosis of fascioliasis in serum and milk samples was performed using the Monoscreen Ab F. hepatica ELISA kit (Bio-X Diagnostics). Milk samples were diluted 1:50, and serum samples were diluted 1:100 in accordance with the kit instructions and the procedure described

by **Harlow and Lane (1988).** The diluted samples were incubated with horseradish peroxidase-conjugated antibodies specific for *Fasciola hepatica* antigens. After incubation, the reaction was developed, and optical density was measured to determine antibody presence and levels.
This indirect ELISA protocol enables sensitive and specific detection of antibodies against *Fasciola hepatica* in both serum and milk, facilitating diagnosis and monitoring of infection status**.**

Analyses of Milk Samples
Total Mesophilic Count
Serial decimal dilutions were prepared following the ISO International Organization standards (2017). Initially, 9 ml of sterile 0.1% peptone water was mixed with 1 ml of milk to achieve a 1:10 dilution, which was homogenized by shaking for 5–10 seconds. Subsequently, 1 ml of this initial dilution was transferred into 9 ml of sterile 0.1% peptone water to obtain further dilutions. Aliquots (1 ml) of the original samples and serial tenfold dilutions were plated onto standard plate count agar (Oxoid, CM0463) as per ISO (2013). Plates were incubated aerobically at 30°C for 72 hours before colony enumeration. This procedure follows the widely accepted pour plate method used for aerobic mesophilic bacterial counts in milk and dairy products, providing an estimate of the viable bacterial population.

b. Somatic Cell Count (SCC)
Somatic cell counts in milk samples were determined using cell-specific fluorescent dyes analyzed by flow cytometry and microscopy, representing current state-of-the-art methodology for SCC detection in dairy quality assessment **(Ferronatto *et al*., 2018).** This approach allows for rapid, accurate quantification of somatic cells, which are indicators of udder health and milk quality.

c. Compositional Parameters Measurement of Milk
Milk compositional analysis, including protein, fat, lactose, and solids-not-fat (SNF) percentages, was performed using a milk scanning apparatus **(Franke Gerber-type, Gerber, 1998).** This standardized method provides reliable and precise quantification of key milk constituents critical for assessing quality and nutritional value.

Analyses of Serum Samples
Serum Biochemical Analysis:
Serum levels of alanine transaminase (ALT), aspartate aminotransferase (AST), urea, and creatinine were measured using commercial kits supplied by Human Co., Germany, to evaluate liver function and renal status. Oxidative stress markers, including glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), and total antioxidant capacity (TAC), were quantified using commercial kits from Biodiagnostic, Egypt. All biochemical and oxidative stress parameters were determined spectrophotometrically using a 5010 V5+ photometer (RIELE Co., Germany), following the manufacturer's protocols (**Nasreldin and Zaki, 2020).**
Serum levels of glutamate dehydrogenase (GDH) and gamma-glutamyl transferase (GGT) were quantified using enzyme-linked immunosorbent assay (ELISA) kits specific for bovine species (Cloud-Clone Corp., USA). Additionally, serum GGT concentration was determined quantitatively by an enzymatic kinetic assay using a 5010 V5+ photometer (RIELE Co., Germany) and commercial reagents from Pointe Scientific, USA, according to the manufacturers' instructions.

Immunological Studies:
Immunological parameters including interferon -gamma (IFN-γ) and interleukin-4 (IL-4) were measured using bovine-specific ELISA kits (RayBiotech, USA). Levels of interleukin-10 (IL-10) and transforming growth factor-beta 1 (TGF-β1) were determined using corresponding bovine ELISA kits obtained from Cusabio, USA, following established protocols **(Nasreldin and Zaki, 2020).**

Statistical Analysis
Data were analyzed using Student's *t*-test to compare serum biochemical parameters, cytokine profiles, oxidative stress markers, and milk composition between control and infected groups. Differences were considered statisti-

cally significant at $P \leq 0.05$ **(Field, 2013).**

Results

Fecal Examination findings

Characteristic large yellowish oval eggs of *Fasciola hepatica* were identified in the feces of 17 cattle. Repeated fecal examinations conducted over the course of a month detected eggs in an additional 10 samples, bringing the total number of positive samples to 27.

ELISA Test findings

The ELISA test performed on milk and serum samples yielded positive results in the same 27 cattle that tested positive by fecal examination (Fig. 1).

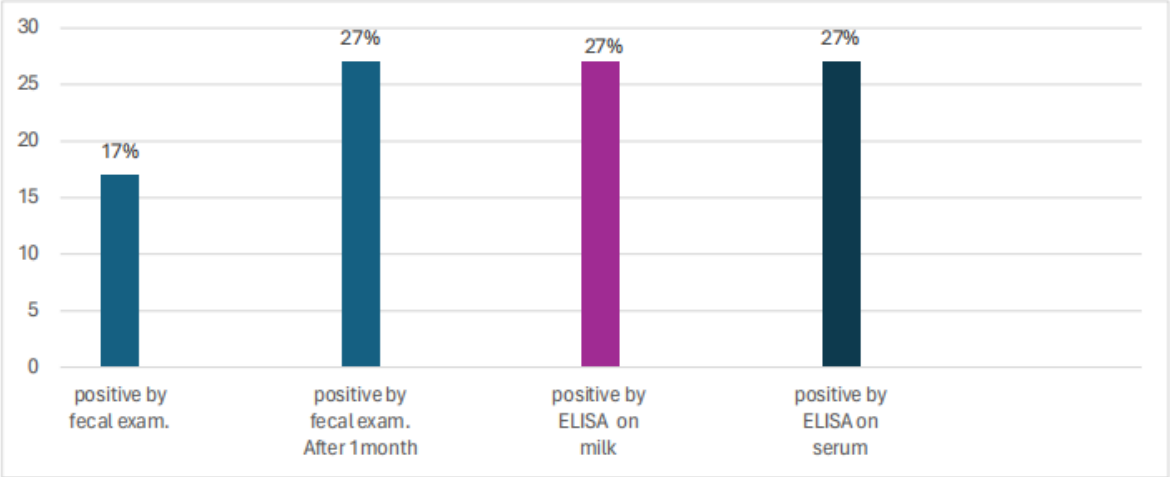

Figure (1). The Positive cases by fecal examination and ELISA test on milk and serum samples.

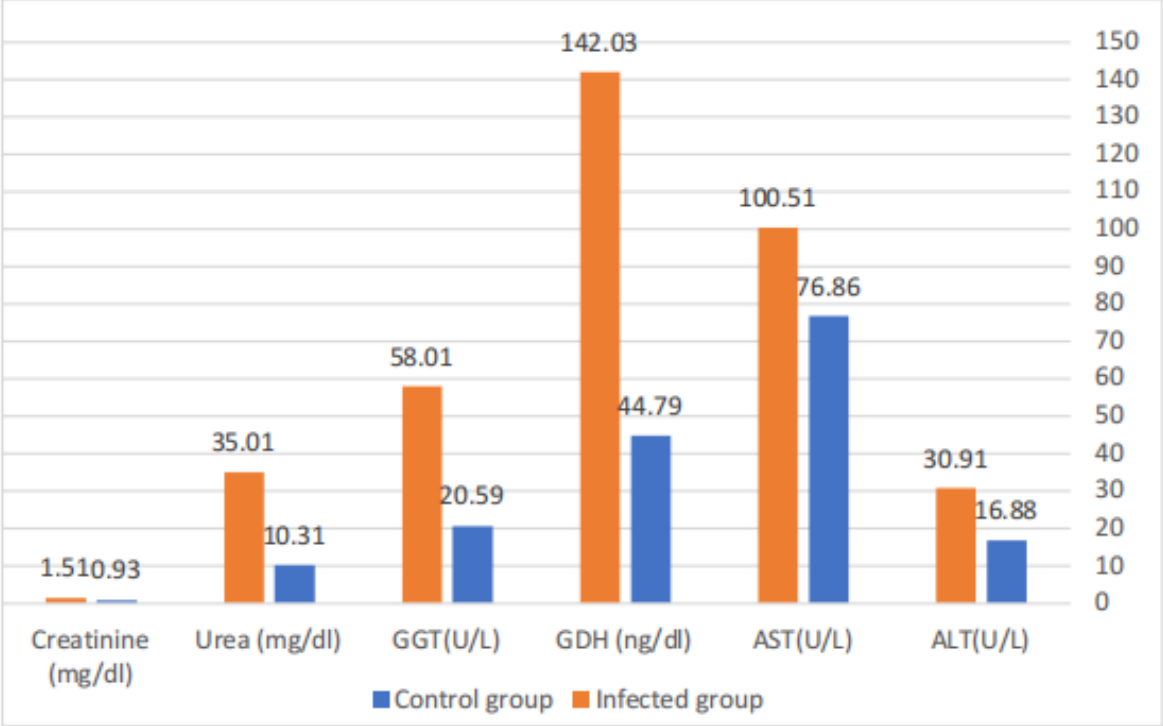

Figure (2). Serum biochemical profiles (Mean± S.E) (control group and infected cattle)

Table (1). Cytokine profiles (Mean± S.E) for control group and infected cattle

Cytokine profiles	Control group	Infected group
IL-4	45.01± 1.02[b]	326.01 ±10.66[a]
IL-10	7.79± 0.09[b]	28.02 ±0.06[a]
TGF β1	11.39 ±0.51[b]	42.12± 0.61[a]
IFN-ɣ	0.83 ± 0.01[a]	0.56 ±0.01[b]

*a, b in the same rae indicate significant differences, p<0.05

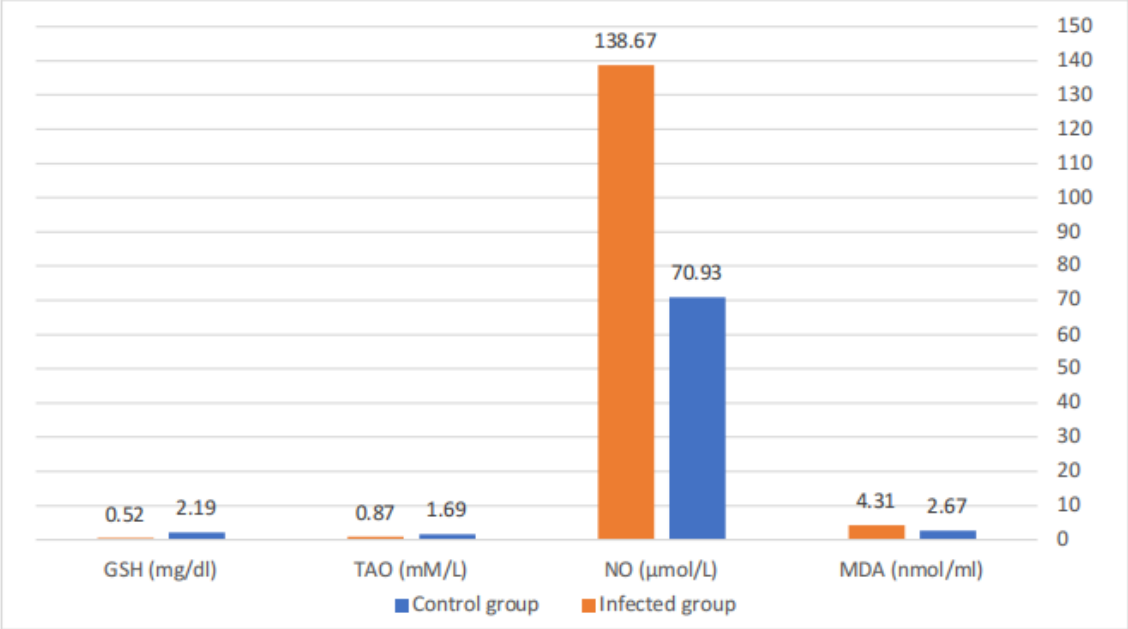

Figure (3). Oxidative stress profiles (Mean± S.E) for control and infected cattle

Table (2). Milk analysis (Mean± S.E) for control group infected cattle

Milk analysis	Control group	Infected group
Total mesophilic count(CFU/ml)	$1.39x10^4 \pm 8.62x10^{2b}$	$2.16x10^6 \pm 1.59x10^5$a
Fat%	3.67 ± 0.23a	2.28 ± 0.21b
SNF%	8.91 ± 0.53a	8.09 ± 0.59b
Protein%	3.6 ± 0.19a	3.12 ± 0.13b
Salts%	0.79 ± 0.13b	0.87 ± 0.08a
Lactose%	4.39 ± 0.22a	3.49 ± 0.3b
pH value	6.65 ± 0.31a	6.89 ± 0.54a
MSCC/ml	$9.31x10^5 \pm 5.32x10^4$b	$1.21x10^6 \pm 9.06x10^4$a

*a, b in the same raw indicate significant differences at p<0.05

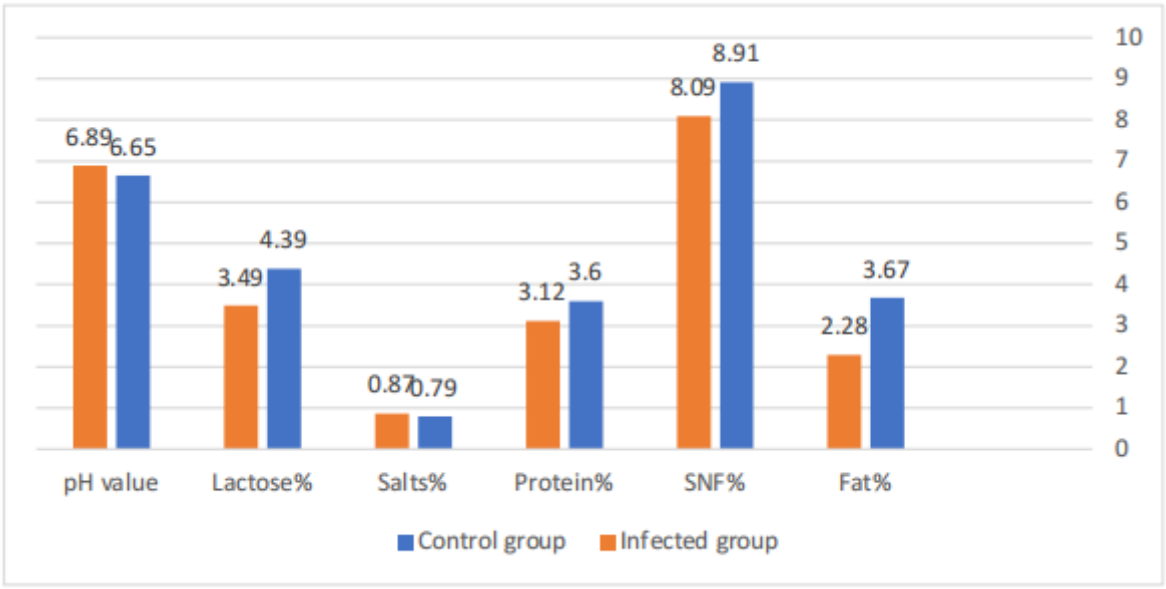

Figure (4). Milk analysis (Mean± S.E) for control group infected cattle

Discussion

Fascioliasis is a pasture-borne parasitic disease commonly diagnosed by fecal examination. While fecal examination is considered accurate and conclusive in positive cases, its sensitivity in the early stages of infection—before flukes begin egg production—or in low-intensity infections remains limited **(George *et al*., 2019; May *et al*., 2019; Corrales *et al*., 2021).** This limitation aligns well with our data, where eggs of *Fasciola hepatica* were initially absent in the feces of 10 cattle at the study's onset, despite these animals testing positive by ELISA in milk. Subsequent repeated fecal examinations one month later confirmed the presence of *F. hepatica* eggs in these cases, illustrating the temporal diagnostic advantage of ELISA for early detection.

The prevalence of *F. hepatica* infection among the studied cattle was 27%, as determined by both fecal examination and ELISA testing on milk samples. This figure corresponds closely to previous reports by **Morsy *et al*. (2005)** in Al-Fayoum Governorate (25.5%), **Haridy *et al*. (2006)** in Gharbia Governorate (21.8%), and **Elshraway and Mahmoud (2017)** in El-Kharga Governorate (30.88%). However, our prevalence is considerably higher than the extremely low rate reported by **Elmonir *et al*. (2015)** in Elmahalla Elkobra Abattoir (0.14%), yet substantially lower than the higher prevalence reported by **Sotohy *et al*. (2019)** in New-Valley Governorate (58.3%), **Pfukenyi and Mukaratirwa (2004)** in Zimbabwe (37.1%), and **Abraham and Jude (2014)** in Nigeria (44.8%). These discrepancies are likely attributable to differences in climatic conditions affecting the intermediate snail host's distribution and survival of infective stages, variable prophylactic strategies, and animal management practices, including diet.

Milk-based ELISA tests offer distinct advantages, including simplicity in collection, ability to store larger volumes for analysis, and non-invasive sampling, making them increasingly favored for farm-level diagnosis and monitoring of fasciolosis **(Bennema *et al*., 2009; Charlier *et al*., 2014).** Our study's data demonstrated significant elevations in hepatic enzymes (ALT, AST, GDH, GGT), as well as urea and creatinine in infected cattle, which corroborates the association between liver inflammation and raised serum enzyme levels described by **Hill *et al*. (2010).** The marked increase in serum GGT levels has been linked to bile duct damage caused by Fasciola spp., specifically hyperplastic cholangitis and biliary epithelial injury **(May *et al*., 2017; Agneessens *et al*., 2000; Bellet *et al*., 2016).** Furthermore, the elevated serum urea and creatinine observed in infected animals suggest renal involvement, potentially explained by

immune complex deposition causing membranoproliferative glomerulonephritis, as reported by **Bennema *et al*. (2010).** Such renal pathology underscores the systemic impact of fascioliasis beyond hepatic tissue.

Our immunological findings reveal a distinct cytokine profile in infected cattle, characterized by significantly increased IL-4, IL-10, and TGF-β1, coupled with a slight reduction in IFN -γ compared to controls (Table 1). This pattern likely reflects an anti-inflammatory, Th2-skewed immune response indicative of chronic infection and immune modulation by *F. hepatica*. **Ersbøll *et al*. (2006) and Mazeri *et al*. (2016)** demonstrated inhibition or low expression of IFN-γ genes in infected hepatic tissues, consistent with our findings. Elevated IL-4 and IL-10 gene expression have similarly been observed in cattle naturally infected with *F. hepatica* **(Bennema *et al*., 2010; Kuerpick *et al*., 2013).** The induction of regulatory cytokines such as IL-10 and TGF-β1 is crucial for limiting immune-mediated pathology while promoting tissue repair and fibrosis, which may encapsulate flukes and prevent hepatic parenchymal invasion, thus enhancing host defense **(May *et al*., 2017).**

Oxidative stress markers also support this pathophysiological narrative: our study found significantly increased levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, in infected cattle. This aligns with prior reports attributing elevated MDA to free radical-mediated oxidative stress induced by Fasciola spp. invasion **(Byrn *et al*., 2016; Morgan *et al*., 2013; Van Dijk *et al*., 2010; Bellet *et al*., 2016; Hill *et al*., 2010).** Such oxidative stress may exacerbate hepatic damage and systemic inflammation in infected cattle.

Milk composition analysis (Table 2) revealed that *F. hepatica* infection is associated with significant reductions in fat, protein, solids-not -fat (SNF), and lactose content, indicating compromised milk quality. This finding echoes **May *et al*. (2019),** who reported lower milk fat and protein percentages in infected cows, and the recent observations of **Oehm *et al*. (2023)** in German Holstein herds. **Dowling *et al*. (2025)** further emphasized the economic repercussions linked to reduced milk fat in infected animals. Contrarily, **Michalski (2002)** reported increased milk protein, fat, lactose percentages, and somatic cell counts in infected cows, highlighting the variability in clinical manifestations that may depend on breed, infection intensity, or management conditions.

The increased total mesophilic counts in milk from infected cows ($1.39\times10^4 \pm 8.62\times10^2$) compared to controls ($2.16\times10^6 \pm 1.59\times10^5$) suggest poorer udder health in affected animals, corroborating the findings of **Mena and Marwa (2021),** who observed elevated bacterial counts in ELISA-positive samples. The slight increase in milk salt percentage in infected cows may be attributable to inflammation-induced alterations in milk secretion.

On the other hand, it is noteworthy that nematode infections have been reported to exert minimal impact on milk yield and composition **(Dank *et al*., 2015),** emphasizing the specific detrimental influence of fascioliasis.

Taken together, these findings underscore the multifaceted impact of *F. hepatica* infection not only on hepatic and immunological health but also on milk quality and farm economics, warranting continued surveillance and incorporation of sensitive diagnostic tools such as milk ELISA for early detection and management.

Conclusions

Fasciola infection acts as a significant stressor on cattle health by activating the immune system and inducing physiological alterations. Moreover, infection leads to changes in the compositional constituents of milk, resulting in reduced milk quality. The ELISA technique demonstrates high specificity and sensitivity for detecting Fasciola spp. antibodies in both serum and milk samples. Notably, milk sampling is less stressful for animals compared to blood collection, making it a practical tool for on-farm surveillance. Early diagnosis and timely treatment guided by milk ELISA can effectively reduce the morbidity and mortality associated with liver fluke infection. This study provides evidence supporting the successful early implementation of milk-based ELISA as a monitoring tool for Fasciola infection in Egyptian cattle.

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