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REGULAR ARTICLES

Evaluation of lactoferrin administration as an antibiotic alternative growth promoter: impacts on rabbit's growth, meat quality, blood biochemical parameters, immune status, antioxidant status, and tissue histology

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Abstract

Antibiotic-resistant microbes have emerged because of the widespread misuse of antibiotics in modern animal production. To overcome antibiotic resistance, the name lactoferrin (Lf) is promising since it is a naturally safe alternative to antibiotics. Considering the few sources available, this study evaluated the effects of Lf administration as a possible alternative to antibiotics on the growth, meat quality, blood hematology, hepato-renal function, antioxidant, and immune status of rabbits. A total of 60 four-week-old V-line male weaned rabbits (average body weight 530±20 g) were randomly distributed to three experimental treatments (TRTs). The first TRT was the control group (CON). In the 2nd and 3rd TRTs, rabbits were orally administered with 1 mL oxytetracycline (OXY)/kg BW and 300 mg Lf/kg body weight (BW), respectively. The TRTs were applied orally twice weekly for 8 weeks. The results showed that the BW of rabbits treated with Lf was higher than the CON rabbits at the 8th and 12th week of age ($P<0.05$). The average daily gain was higher in the Lf-treated group than in the CON and OXY-treated groups in the 12th week ($P<0.01$). The meat quality parameters showed that Lf produced bright red meat with the highest lightness and lowest yellowness and hue values compared to OXY TRT and CON rabbits. At the same time, the other technological meat parameters, such as water holding capacity, drip loss (24 and 48 h), cooking loss, and Warner-Bratzler Shear Force, were not significantly changed. The serum levels of AST, ALT, ALP, and urea were reduced in the Lf-treated group and increased in the OXY-treated group compared with the CON. The total and direct bilirubin levels and creatinine and uric acid levels were increased in the OXY-treated rabbits. The total protein and globulin serum levels were raised in the Lf-treated rabbits and reduced in the OXY-treated rabbits. The albumin level decreased in the OXY-treated group. The serum levels of IgG and IgM were higher in the LF-treated animals compared to the CON and OXY-treated groups ($P<0.01$). The serum levels of IgA, IgD, and IgE were increased in both Lf- and OXY-treated groups compared with the CON group. The hepatic and renal MDA and NO concentrations were decreased in the LF-treated group and increased in the OXY-treated group. The opposite is true for the activity of glutathione peroxidase, glutathione-reduced glutathione-S-transferase, and catalase. Lactoferrin administration upregulated the growth-related genes and downregulated the proinflammatory and apoptotic genes. In conclusion, lactoferrin in a dose of 300 mg /kg BW is a potential antibiotic alternative for improving rabbits' growth, health, meat quality, antioxidant, and immune status.

Keywords Rabbit · Meat quality · Health · Antioxidant/immune status

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Introduction

Globally, persistent antibiotic use and abuse in agriculture and animal sectors has led to higher antibiotic residues in animal products. These, combined with the high transferability of resistant genes, caused an increase in the emergence of antibiotic-resistant microbes in meat (Koo and Woo 2011; Schwarz et al. 2001), which is a significant public health problem that necessitates the advancement of new antimicrobial compounds. Rabbits are hindgut fermenters that digest plant-based diets based intensely on their gastrointestinal microbiota. Accordingly, antibiotics should be used cautiously in rabbits as disruption of the normal intestinal flora permits the proliferation of pathogenic bacteria such as Clostridia Sp or coliforms with their ensuing toxins (Wheler 2013).

Lactoferrin (Lf), derived from animals, is one of these promising natural substances. Lactoferrin, a safeguard multipotential and multifunctional cationic glycoprotein, comprises amino acids in a single homologous polypeptide chain among mammalian species. It is one of the iron-binding transferrins (TF) where its chelated capacity is 300-fold greater than TF, has an immunological reaction in the innate immune defense, and has high iron binding stability even at acidic values of 3.0 pH (Wang et al. 2019). Lf is a non-haem cationic mammalian glycoprotein, 78–80 kDa in size, found in several body secretions, such as respiratory, gastrointestinal, and reproductive. Bovine Lf (bLf) is found in high levels in colostrum (2–5 mg/ml) and lower levels in mature milk (0.1–0.3 mg/ml) (Inoue et al. 1993; Pan et al. 2007). Salivary-produced Lf is correlated with host defense against pathogenic organisms in the mouth. In addition, Lf is formed by secondary granules of neutrophils, which produce this protein at sites of infection (Vogel 2012).

Lactoferrin has several biological purposes involving antibacterial activity, iron absorption regulation, and macrophage growth promotion (Sakai 1999). It is a safe and powerful nutraceutical and pharmaceutical alternative protein to chemotherapy and is promptly commercially available (Jia et al. 2021; Luna-Castro et al. 2017). Oral administration of bLf has increased host defense against infection through immunomodulation and antimicrobial action (Tomita et al. 2009). Lactoferrin possesses several physiological properties such as antioxidant activities (Brock 2012; Kanwar et al. 2015), immunomodulatory (Siqueiros-Cendón et al. 2014), antimicrobial (Embleton et al. 2013), antiviral (Berlutti et al. 2011), antifungal, and antiparasitic (Leboffe et al. 2009). Lactoferrin is an essential innate immunity component (Borodina et al. 2020). Lactoferrin supplementation changed the gut microbiota conformation and diversity, involving a rise in the beneficial bacteria, for example, *Lactobacillus and Bifidobacterium*, and a reduction in pathogenic bacteria such as *Enterobacteriaceae* (Konstanti et al. 2022).

Lactoferrin bioavailability is essential when Lf is used as a dietary supplement to treat infectious diseases. Lactoferrin outer shell has been reported to improve lactoferrin absorption in the small intestine (Kawakami et al. 2015). In addition, lactoferrin can modify physiological functions, maturation, and migration of immune cells (Legrand et al. 2005). The US Food and Drug Administration (FDA) has authorized the potential of Lf as a safe, natural antimicrobial material for different food classes (Taylor et al. 2004).

Lactoferrin has established a wide range of biological activities, involving immunomodulatory, antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and antiosteoporosis and associated diseases. So, lactoferrin can be a suggesting applicant for enhancing the health and production efficiency of livestock. Lactoferrin is used as a nutritional supplement for livestock and other food animals to promote growth, productivity, and overall health. Furthermore, lactoferrin improves the digestion and absorption of nutrients (Behan et al. 2024). Recently El-Sharawy et al. (2024) showed increased daily weight gain, hemoglobin concentration, and total leukocyte count of the goat kids treated with Lf (50–200 mg/ml) compared to the control group during the weaning phase. They also reported higher total protein, albumin, and creatinine levels by Lf administration, especially at level 200 mg/ml than the control group. Previous research works have concentrated on the effect of Lf after direct application on the quality of meat products (Heller et al. 2007; Taylor et al. 2004), with little concern for the consequences of dietary Lf on meat quality and other health aspects. Consequently, this study estimated the effects of lactoferrin administration as an antibiotic alternative on the growth, meat quality, blood hematology, blood biochemical parameters, tissue histology, and immune and antioxidant status of rabbits.

Materials and methods

Lactoferrin and antibiotic used

Lactoferrin (Cat. No. EXTC-135) was obtained from creative enzymes (65830 Kriftel, Gutenbergstraße 5. Frankfurt am Main, Germany). Oxytetracycline was bought from Adwia Pharmaceuticals, Cairo, Egypt. All chemicals and kits used in this study were analytical grade.

Animals and experimental design

This investigation was done at the Rabbit Research Unit, Faculty of Agriculture, Benha University, Egypt. Animals

were reared according to husbandry standards derived from Benha University Standard Operating Procedures. A total of 60 four-week-old V-line male weaned rabbits (average body weight 530 ± 20 g) were distributed into three experimental TRTs ($n = 20$) and individually housed in metabolic cages (45 × 55 × 30 cm) with manual feeders and drinkers. The animals were fed *ad libitum* and were kept for a two-week adaptation period before the experiment. The first TRT was the control group (CON), while the other two TRTs were orally administered with oxytetracycline (OXY) at 1 mL/kg BW (equivalent to 200 mg/kg BW) and lactoferrin (Lf) at 1 mL/kg BW (corresponding to 300 mg/kg BW), respectively. The TRTs were administered orally twice weekly for 8 weeks. During the experimental period, rabbits were fed the same standard iso-caloric/iso-nitrogenic diet. The basal diet composition and analysis were performed following NRC (1966), as shown in Table 1. During the experiment, the rabbits were housed in an open system with an average temperature of 39.2 °C, a relative humidity of 55–60%, and a 16–8 h light-dark cycle. The animals were weighed at the 4th, 8th, and 12th weeks of age, and the average daily weight gain (g) was determined subsequently as the No of grams gained between two weights/ animal/ No of days.

Table 1 Composition and chemical analyses of the basal diet (g/kg, as-fed basis)

Ingredients	g/kg
Yellow corn	110
Alfalfa hay	330
Soybean meal	96.9
Wheat bran	300
Barely grain	90
Wheat straw	50
DL-Methionine	2.3
L-Lysine HCl	1.8
Di-calcium phosphate	12.5
Vitamin/mineral premix[1]	1.5
Sodium chloride	5
Total	1000.0
Chemical analysis	
Dry matter	914.41
Crude protein	181.79
Ether extract	30.23
Digestible energy (MJ/kg)	10.37
Crude fiber	135.36
Ash	65.30
Ca	10.74
Available Phosphorus	5.94
Lysine	9.08
Methionine	4.34

[1] Supply kg^{-1}: Vit. A: 15,000 IU, Vit. D3: 2,500 IU, Vit. E: 16.66 mg, Vit. K: 2.0 mg, Vit. B1: 1 mg, Vit. B2: 4 mg, Vit. B6: 1.66 mg, Vit. B12: 0.0034 mg, pantothenic acid: 6.66 mg, biotin: 1.07 mg, folic acid: 1.66 mg, choline chloride: 400 mg

Sampling

At the end of the experiment, ten rabbits from each treatment group underwent anesthesia via intramuscular injection of ketamine and xylazine. Subsequently, 10 ml of blood was drawn from the ear vein of each rabbit into EDTA-containing tubes (K3EDTA; Sigma Company, St. Louis, MO, USA). These blood samples were refrigerated and promptly utilized for hematological parameter assessment following Schalm (1962). Additional blood samples were collected in sterile tubes without anticoagulants, centrifuged at 3000 rpm for 10 min, and the resulting serum was stored at -20 °C for subsequent biochemical tests. Following blood collection, rabbits ($n = 10$) were sacrificed following an 8-hour fast (Association 2013). Muscle samples from the right and left *Longissimus lumborum* were collected to analyze the meat quality. The Liver and kidney samples (10 samples/group) were collected and homogenized to evaluate oxidant/antioxidant indices. Samples from the intestine and liver (10 samples/group) were preserved in 10% buffered formalin to determine the histo-morphological indices and histopathological analysis.

Meat quality measures

Right and left *Longissimus lumborum* (LL) cuts were dissected from rabbits carcasses to determine pH, water holding capacity (WHC), drip loss (24 and 48 h), and cooking losses (samples taken 15 min to reach 75 °C in preheated water bath), Warner-Bratzler Shear Force (WBSF), lightness (L *), redness (a *), yellowness (b *), chroma (C), and Hue angle (h) as described earlier (Elokil et al. 2019; Imbabi et al. 2021).

The keeping quality of the rabbit meat was evaluated over 10 days, as stated previously for rabbit and chicken meat (El-Bahr et al. 2020; Osman et al. 2021), where the hind legs were immediately dissected from the rabbit carcasses under aseptic condition, the bone was trimmed, the meat portions from various replicates of the same group were minced together, and then 25 g of the meat homogenate were loaded into a sterile falcon tube (50 ml). Three falcon tubes were distributed for each checkpoint (5 checkpoints; day 1, day 3, day 5, day 7, and day 10) and afterward placed in a programmable incubator (Binder KB 23, BINDER GmbH (Headquarters), Tuttlingen, Germany) at 5 ± 0.2 °C for further determination of APC and pH. APC was determined in the same manner as previously demonstrated for natural beef microflora (Sabike et al. 2015), while pH was measured by the direct insertion of a pH-meter glass electrode into minced meat within the Falcon tube.

Blood hematology

The SYSMEX hematology auto analyzer (Japan) performed hematological analysis, which included estimating white blood cell (WBC), red blood cell (RBC), platelet counts (PLT), hemoglobin concentration (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Liver and kidney function biomarkers and proteinogram

Alanine amino transaminase (ALT), aspartate amino transaminase (AST), alkaline phosphatase (ALP), and liver total, direct and indirect Bilirubin were determined according to Reitman and Frankel (1957) and Tietz (1983). Total protein and serum albumin levels were measured according to Doumas (1975). Globulin was calculated by subtracting the albumin from serum total protein. Urea, uric acid, and creatinine levels were measured according to Tabacco et al. (1979).

Histopathological assays

Intestinal and liver samples ($n=9$) were collected, fixed in 10% buffered formalin, dehydrated using ascending grades of ethyl alcohol (70–100%), cleaned in xylene, and then embedded in paraffin. Paraffin slices (5 µm thickness) were cut with a microtome (Leica RM 2155, England). Hematoxylin and eosin stain (H&E) were used to stain the sections (Bradford 1976). The stained sections were inspected with the Ceti England microscope with an associated AmScope digital camera for histopathological analysis (Suvarna et al. 2013).

Immunoglobulin levels

Measurements were conducted using the ELISA device to determine the concentrations of serum immunoglobulins (IgA, IgE, IgG, IgD, and IgM) from the obtained serum sample with a commercial abcam immunoglobulin test kit.

Oxidative stress and antioxidant status

Liver and kidney specimens were washed with saline solution to remove blood, dried by blotting with filter paper, and weighed. Preparation of liver homogenates (10% w/v) by homogenizing tissue in 100×10^{-3} M potassium phosphate buffer (pH 7.4), then centrifuged at 1500 xg for 10 min at 4 ˚C (Centurion Scientific Ltd, K2015R, UK). Oxidative stress was estimated by measuring the concentration of Nitric Oxide using the method of Montgomery and Dymock (1961). Lipid peroxides (malondialdehyde, MDA) were measured according to Uchiyama and Mihara (1978). Antioxidant enzyme activity such as glutathione peroxidase (GPX) was assayed following Flohé and Günzler (1984), and glutathione-S-transferase was performed according to Habig et al. (1974). Glutathione reduced as described before by Prins et al. (1969). Catalase activity was performed according to the method of Böck et al. (1980).

Quantitative real-time PCR analysis (qRT-PCR)

According to the manufacturer's instructions, each TRT group's total RNA was extracted from 50 mg tissues (liver and caecum) with 1 mL Quiazol (Qiagen, Germany). The cDNA synthesis and the qPCR were done as described by Khamis et al. (2021). The RT-qPCR analyses of the tumor necrosis factor-alpha *(TNF-α)*, Glutathione peroxidase *(GPX)*, growth hormone *(GH)*, fatty acid desaturase*) FADS2A*, *FADS2G(*, *FAS*, acyl-coenzyme A oxidase 1 *(ACOX1)*, *and* melanocortin 4 receptor *(MC4R)* were performed with specified primers (Sangon Biotech, Beijing, China) using beta-actin (β-actin) as a reference gene as listed in Table 2 as previously described (Schmittgen and Livak 2008).

Statistical analysis

The statistical analysis was performed using a completely randomized design and the general linear model (GLM) procedure of SAS 9.2. All data were evaluated for normal distribution ($W>0.05$) using the Shapiro–Wilks test. Then, one-way ANOVA was performed using SAS 9.2 (Institute 2009) statistical software. Post-hoc Tukey's test was used to determine differences among means. The variation in the data was expressed as pooled standard error mean (SEM) and the significance level was set at $P\leq0.05$.

Results

Growth performance

Table 3 shows the changes in the body weight and average daily gain of rabbits treated with different treatments. In the 4th week, there were no significant changes in the body weights of animals on the different TRTs ($P>0.05$). In the 8th and 12th weeks, the BW of animals treated with Lf was higher than the CON rabbits ($P<0.05$). In the 8th week, the average daily gain (ADG) was higher in the Lf-treated group than in the CON group ($P=0.01$). At the 12th week,

Table 2 Primer design for genes analyzed by real-time PCR

Gene	Primer sequences	Accession number	Product size (bp)
TNFα	F: CAGCCTTGTCCCTTGAAGAGAGAACC R: TACTGAACTTCGGGGTGATTGGTCC	M_35326	220
FADS2A	F: AAATCCTGCCGCAGAGAAG R: TCGCACATAGCTCCGTGTT	NM_019699	100
GPX	F: TTACGCTCCCATTCAGAAGC R: TTGTAAACATCAGGGGCAAA	Z_38127	239
ACOX1	F: ATGCTGATGAAACATGCCCAGGTG R: TTCAGACTGATGCCTCACAGCACT	XM_020915575	364
FADS2G	F: AACCTTCCGCTCTATCACCA R: GGGCCGACGTTGCCGCG	XM_046918286	470
FAS	F: AAGCTGAAGGCTGCTGACAAGT R: CCTCCAATAAGGTGCGGTGAT	NM_205155.4	184
MC4R	F: GCAATTGCTGTGCAGTCCATA R: CAACCCCAGTTACCAGCACT	HF_970577	417
GH	F: TTACGCTCCCATTCAGAAAGC R: TTGTAAACATCAGGGGCAAA	Z_38127	239
β actin	F: GTGGGGCGCCCCAGGCACCA R: CTCCTTAATGTCACGCACGATTTC	XM_054496084	187

Beta-actin (*β-actin*), tumor necrosis factor-alpha *(TNF-α)*, Glutathione peroxidase *(GPX)*, growth hormone *(GH)*, fatty acid desaturase*) FADS2A*, *FADS2G(*, *FAS*, acyl-coenzyme A oxidase 1 *(ACOX1)*, melanocortin 4 receptor *(MC4R)*

Table 3 Growth performance of rabbits in the different experimental TRTs

Item	CON	OXY	Lf	SEM	*P*-value
BW(4 w)	533.57	549.07	549.10	53.43	0.972
BW (8 w)	873.50^{b}	973.33^{ab}	1030.00^{a}	32.08	0.035
BW (12 w)	1402.87^{b}	1612.13^{ab}	1667.38^{a}	64.44	0.045
ADG (4–8 w)	12.14^{b}	15.15^{ab}	17.17^{a}	1.37	0.0102
ADG (8–12 w)	18.90^{b}	22.81^{a}	22.76^{a}	1.59	0.0210

$^{a, b}$ Mean values in the same row with different superscripts differ significantly ($P<0.05$). BW, body weight; ADG, Average daily gain

Table 4 Meat quality parameters of *Longissimus lumborum* (L.L.) cuts of rabbits of the different TRTs

Parameters	CON	OXY	Lf	SEM	*P*-value
pH (24 h)	5.87	5.97	5.91	0.02	0.21
WHC	90.06	90.32	90.17	0.28	0.94
Drip loss (24 h) %	1.45	3.38	1.96	0.41	0.13
Drip loss (48 h) %	4.42	4.39	3.56	0.27	0.38
Cooking loss %	14.22	17.73	9.69	2.26	0.40
WBSF	4.80	4.38	4.81	0.21	0.67
*L**	51.01 ^{b}	47.78 ^{c}	52.04 ^{a}	0.56	0.00
a*	15.39 ^{b}	17.95 ^{a}	16.12 ^{b}	0.40	0.01
b*	7.80 ^{a}	7.88 ^{a}	6.79 ^{b}	0.19	0.02
Chroma	17.25 ^{b}	19.60 ^{a}	17.50 ^{b}	0.41	0.01
Hue (h°)	26.87^{a}	23.72 ^{b}	22.83 ^{b}	0.57	0.00

$^{a, b}$ Mean values in the same row with different superscripts differ significantly ($P<0.05$). Water holding capacity (WHC), Warner-Bratzler Shear Force (WBSF), lightness (L *), redness (a *), yellowness (b *)

the ADG was increased in animals treated with Lf and OXY ($P<0.021$).

Meat quality measures

Table 4 shows the differences in rabbit meat quality caused by lactoferrin supplementation compared to control and antibiotic-fed rabbits. The majority of meat quality changes were linked to color parameters such as redness (a*), yellowness (b*), lightness (L*), Chroma (C), and hue angle (h) ($P<0.05$), while other investigated technological and sensory parameters such as pH24, WHC, drip loss (24 h), drip loss (48 h), cooking loss, and Warner-Bratzler Shear Force (WBSF) were comparable ($P>0.05$). The color results showed that Lf-treated rabbits produced bright red meat with the highest L*, medium a*, lowest b*, and h° values compared to CON and antibiotic-treated rabbits ($P<0.05$). In comparison, meat from OXY-treated rabbits has a lower L* value and higher a*, b*, and h° values ($P<0.05$) than CON and Lf-treated rabbits, indicating darker reddish meat. On the other hand, APC and pH values obtained from different checking days during the keeping quality test of meat from Lf-treated rabbits were often lower than those of meat from CON and OXY groups ($P<0.05$) (Fig. 1, A&B). The initial and seventh-day APC and pH values of Lf-treated rabbit meats were 4.54 log CFU/g and 5.91, and 5.93 log CFU/g and 6.30, respectively, whereas antibiotic-fed rabbit meat had values of 4.89 log CFU/g and 5.97, and 6.39 log CFU/g and 6.44, respectively.

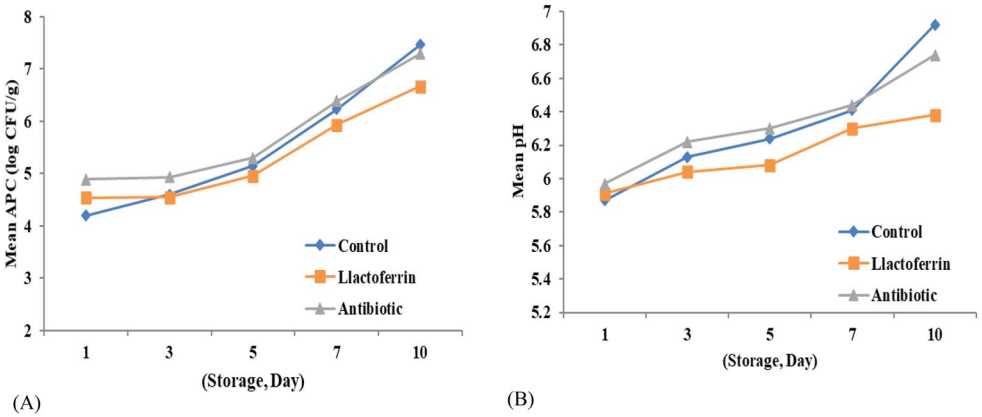

Fig. 1 Aerobic plate count (**A**) and pH value (**B**) of rabbit meat stored at 4 °C for 10 days

Table 5 Blood hematology of rabbits in the different experimental TRTs

Item	CON	OXY	Lf	SEM	*P*-value
RBCs (×10^6/uL)	4.38^b	3.99^c	4.98^a	0.089	0.0007
Hb (g/dL)	12.38^b	9.30^c	13.65^a	0.230	0.0001
WBCs (×10^3/uL)	8.73^b	7.93^c	9.43^a	0.150	0.0012
HCT (%)	36.68^b	34.69^c	38.80^a	0.302	0.0002
MCV (fL)	86.14^b	81.14^c	88.83^a	0.615	0.0003
MCH (Pg)	27.44^b	24.33^c	29.02^a	0.362	0.0003
MCHC (g/dL)	31.34^b	27.16^c	35.54^a	0.789	0.0009

$^{a, b}$ Mean values in the same row with different superscripts differ significantly ($P<0.05$). RBCs, Red blood cells count; Hb, hemoglobin; WBCs: white blood cells; HCT, hematocrit value; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration

Table 6 Liver and kidney function biomarkers and proteinogram in rabbits of the different experimental TRTs

Item	CON	OXY	Lf	SEM	*P*-value
AST (U/L)	31.00^b	66.33^a	28.00^b	2.41	0.0001
ALT (U/L)	39.66^b	80.66^a	35.00^c	1.18	0.0001
ALP (U/L)	90.49^b	115.38^a	86.01^b	1.98	0.0001
Total Bilirubin (mg/dL)	1.10^b	1.73^a	1.00^b	0.033	0.0001
Direct Bilirubin (mg/dL)	0.24^b	0.36^a	0.21^b	0.018	0.0030
Indirect Bilirubin (mg/dL)	0.86^b	1.37^a	0.79^c	0.019	0.0001
Urea (mg/dL)	27.27^b	40.40^a	23.06^c	0.741	0.0001
Creatinine (mg/dL)	0.88^b	2.13^a	0.81^b	0.070	0.0001
Uric acid (mg/dL)	5.93^b	8.63^a	5.46^b	0.317	0.0008
Total protein (g/dL)	8.04^b	5.79^c	8.65^a	0.085	0.0001
Albumin (g/dL)	4.08^a	3.61^b	4.36^a	0.092	0.0036
Globulin (g/dL)	3.96^b	2.36^c	4.28^a	0.073	0.0001

$^{a, b}$ Mean values in the same row with different superscripts differ significantly ($P<0.05$). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, Alkaline phosphatase

Blood hematology

The hematological parameters (RBCs, Hb, WBC, HCT, MCV, MCH, and MCHC) were increased in the Lf-treated rabbits and decreased in the OXY-treated rabbits compared with the CON group ($P<0.01$) (Table 5).

Blood biochemical parameters

As shown in Table 6, the serum levels of AST, ALT, ALP, and urea were decreased in the Lf-treated rabbits and increased in the OXY-treated rabbits compared with the CON group ($P<0.01$). The total and direct bilirubin levels were increased in the OXY-treated rabbits ($P<0.01$), while not changed in the LF-treated rabbits compared to the CON rabbits. The indirect bilirubin level was increased in the OXY-treated rabbits and decreased in the Lf-treated rabbits compared to the CON rabbits ($P<0.01$). The creatinine and uric acid levels were increased in the OXY-treated rabbits compared with the CON group ($P<0.01$). The total protein and globulin serum levels were increased in the Lf-treated rabbits and reduced in the OXY-treated rabbits compared with the CON group ($P<0.01$). The albumin level was decreased in the OXY-treated group compared with the CON group ($P<0.01$).

Histopathological findings

Examined intestinal and liver sections from the CON rabbits showed normal histomorphology (Fig. 2). The OXY-treated group showed hepatic changes with a minor morphological abnormality as represented by focal hepatocellular hydropic degeneration, mild vascular dilatation, and biliary proliferation. The intestines were normal apart of focal villous epithelial stratification. The intestinal folds appeared short and broad compared to the control's. The submucosa showed mild edematous change (Fig. 3). The Lf-treated group pointed out normal histo-morphological counterparts with preserved architectures of liver and intestinal tissues. In a

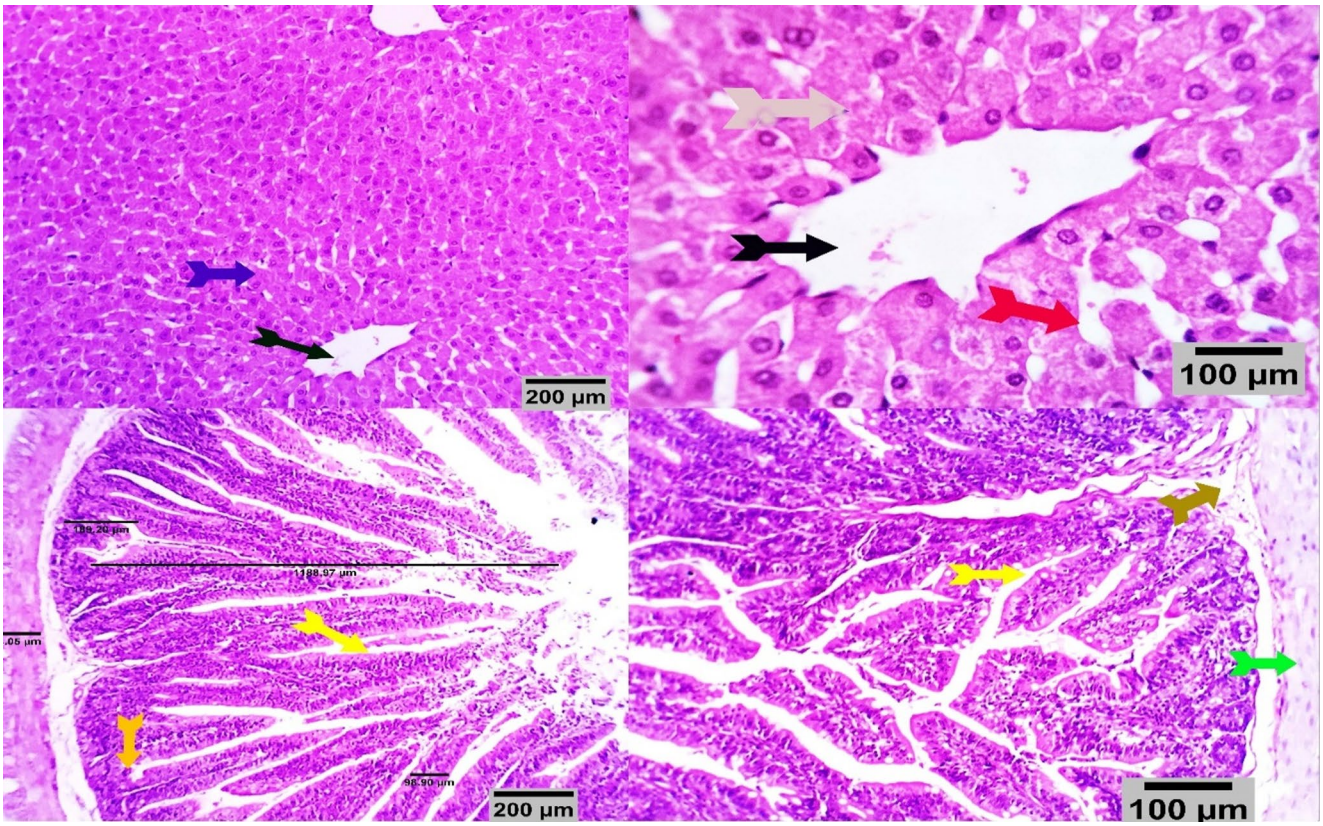

Fig. 2 Photomicrograph of liver and intestine (control group) showing normal hepatic parenchyma with preserved lobular pattern (blue arrow), central veins (black arrow), sinusoids (red arrow), and hepatic cords arrangement (gray arrows). The intestines showed normal histomorphology of the examined segments with preserved villous structures (yellow arrow), intestinal folds, crypt, and glandular structures (orange arrow), submucosal vascular and lymphoid architectures (brown arrow) and normal muscular coat histomorphology (green arrow)—scale bars 1000, 200 µm

few liver sections, mild vascular dilatation was noted. The Von-Kupffer cells appeared reactive hypertrophic. Neither degenerative, apoptotic, or necrotic changes were seen. The intestines in almost all examined sections were normal in terms of the villous, crypt, glandular, vascular, lymphatic, and muscle coat histo-architecture (Fig. 4).

Immune status

The serum levels of IgG and IgM were higher in the Lf-treated animals compared to the CON and OXY-treated groups ($P<0.01$). However, the IgM level was higher in the OXY-treated group than in the CON group ($P<0.01$). The serum levels of IgA, IdD, and IgE were increased in both Lf- and OXY-treated groups compared with the CON group ($P<0.05$) (Table 7).

Oxidative stress and antioxidant status

The hepatic and renal MDA and NO concentrations were decreased in the Lf-treated group and increased in the OXY-treated group ($P<0.01$). The activity of CAT, GSH, GPX, and GST in the hepatic and renal tissues was increased in the Lf-treated group and decreased in the OXY-treated group compared with the CON group ($P<0.01$) (Table 8).

Expression of growth-related genes

The expression of *FADS2G*, *ACOX1*, and *MC4R* genes in the liver and caecum were upregulated in the Lf-treated group compared with the OXY-treated group and the CON ($P<0.05$). The expression of *GH* and *FADS2* genes in the liver were higher in the OXY-treated group than the Lf-treated group, while the opposite was found in the caecum ($P<0.05$). Compared to CON, both Lf and OXY administration upregulated *GH* expression in the liver and caecum (Fig. 5-A, B).

Expression of proinflammatory, apoptotic, and antioxidant genes

The expression of *TNF-α* and *FAS* genes in the liver and caecum were upregulated in the OXY-treated group and downregulated in the Lf-treated group compared to the CON

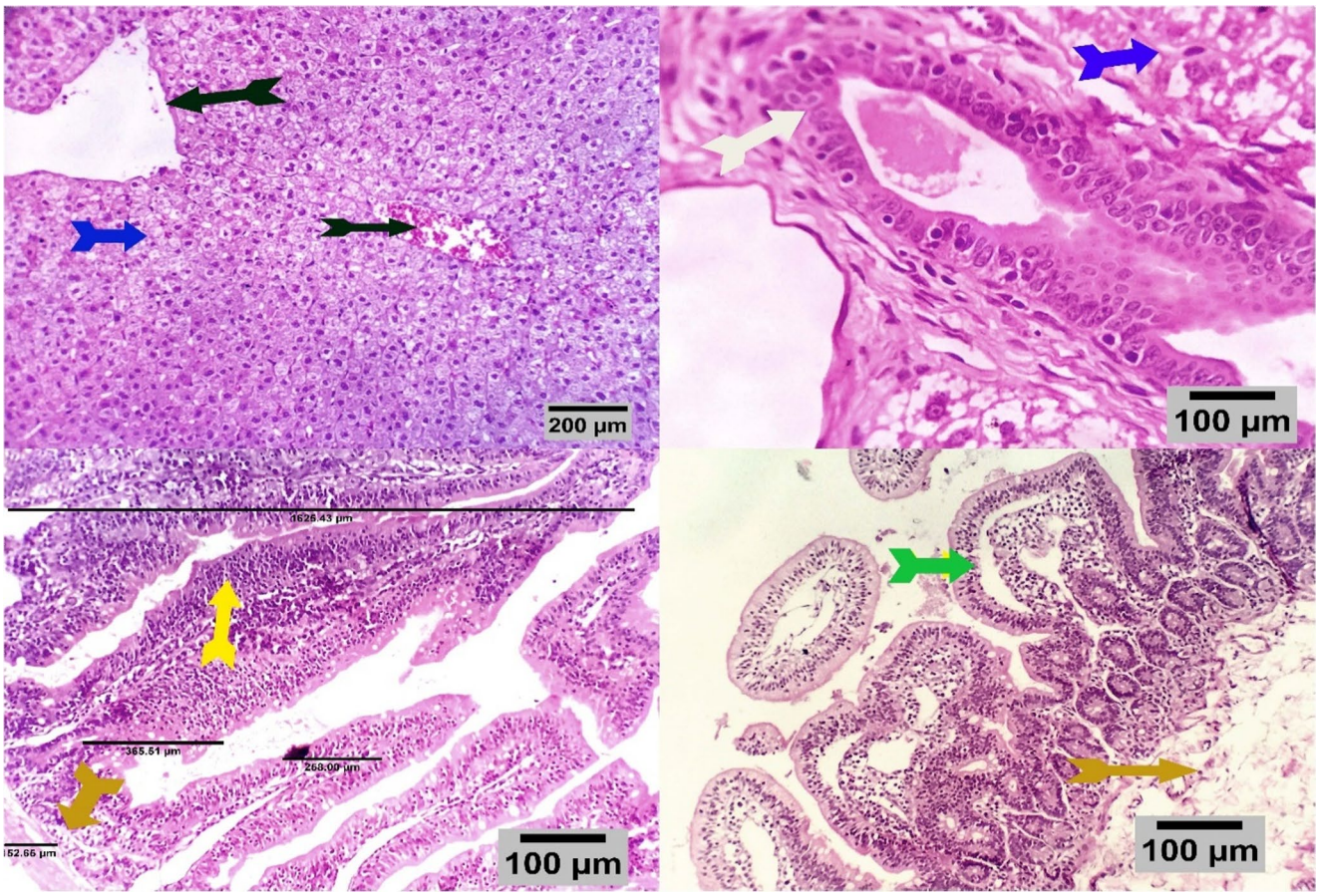

Fig. 3 Photomicrograph of liver and intestine of the OXY-treated group showing focal hepatocellular hydropic degeneration (blue arrows), mild vascular dilatation9 black arrow) and biliary proliferation (white arrow). Intestines show focal villous epithelial stratification (yellow arrow). The intestinal folds appeared short and broad (green arrow). The submucosa shows mild edematous change (brown arrow). Scale bars 100 µm, 200 µm

($P<0.05$). The expression of the *GPX* gene in the liver and caecum was upregulated in the Lf TRT and downregulated in the OXY TRT compared to the CON ($P<0.05$) (Fig. 5A, B).

Discussion

Lactoferrin has numerous diverse biological functions (Superti 2020) that are usually used in the food industry and the health sector (Niaz et al. 2019). The current study showed that the BW of rabbits treated with Lf was higher than the CON rabbits at the 8th and 12th week of age, and the average daily gain was higher in the Lf-treated group than the CON and OXY-treated groups at the 8th week. The improved growth performance of Lf-treated rabbits can be attributed to the upregulation in the expression of *GH*, *FADS2G*, *FADS2A*, *ACOX1*, and *MC4R* genes in the liver and cecal tissues. Pituitary GH advocates *IGF-1* release from the liver, promising somatic growth (Björnsson et al. 2002). The conversion of linolenic acid (C18:2n-6) and alpha-linolenic acid (C18:3n-3) into long-chain polyunsaturated fatty acids, LCP (⩾20 carbons) mainly depends on expression and activity of desaturase (FADS2 and FADS1) and elongase (2 and 5) enzymes, which are similar for both n-3 and n-6 fatty acid series (Barceló-Coblijn and Murphy 2009; Gregory et al. 2011). The FADS2 is the rate-limiting enzyme in the synthesis of arachidonic acid (AA; 20:4n-6), EPA (20:5n-3), and DHA (22:6 n-3) acids from their precursors (Cho et al. 1999). The expression of FADS2 mRNA in the liver and caecum proves its role in LCP metabolism, consequently improving growth and immunity. FADS2 metabolism is commonly altered by feed, substrate competition, genetic strain, and body tissue (Mattioli et al. 2019). ACOX1 is the rate-limiting enzyme in the peroxisomal fatty acid β-oxidation pathway (Zhang et al. 2024). MC4R controls feed intake and satiety, which in sequence affects body weight (El-Sabrout and Aggag 2017). Similarly, these authors found upregulated expression of the *MC4R* gene in high weighed rabbits. Fontanesi et al. (2013) recognized

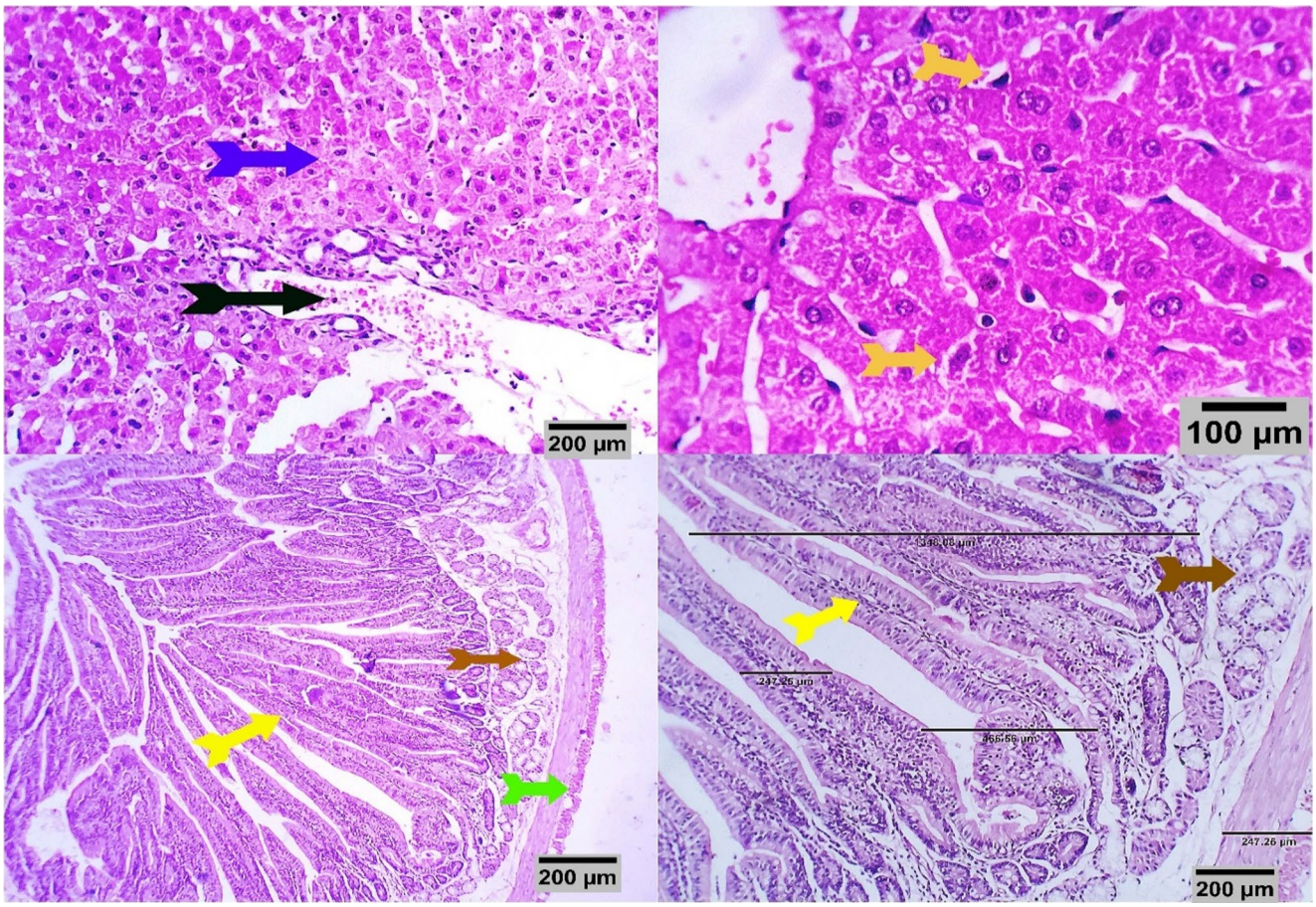

Fig. 4 Photomicrograph of live and intestine of the Lf-treated group showing mild hepatic vascular dilatation (black arrow) and Von-Kupffer cells reactive hypertrophic change (orange arrows). The intestines appear normal in terms of villous (yellow arrow), glandular (brown arrow), and muscle coat histoarchitecture (brown arrow). Scale bars 100 µm, 200 µm

Table 7 Immunoglobulins levels in rabbits of the different experimental TRTs

Item (µg/mL)	CON	OXY	Lf	SEM	*P*-value
IgG	155.70^{b}	165.50^{b}	261.33^{a}	9.84	0.0005
IgM	34.17^{c}	75.53^{b}	93.10^{a}	7.74	0.0044
IgA	53.80^{b}	115.30^{a}	133.90^{a}	8.74	0.0015
IgD	154.33^{b}	252.66^{a}	269.33^{a}	11.08	0.0007
IgE	141.03^{b}	164.00^{a}	165.00^{a}	4.88	0.0220

$^{a, b}$ Mean values in the same row with different superscripts differ significantly ($P<0.05$)

numerous MC4R polymorphisms related to finishing weight in meat rabbit lines.

In the current study, the results recorded for technological parameters and tenderness of meat were not different, though the meat from rabbits treated with OXY as growth promoters were inferior for drip loss (24 h) and cooking losses compared to CON and Lf-treated rabbits. Compared to the CON rabbits, Lf administration resulted in a high lightness value, low yellowness and hue values, and slightly higher redness and chroma values, indicating appealing bright red rabbit meat. The meat color parameters obtained from OXY-treated rabbits, low lightness and high yellowness, redness, and hue, suggest that it will be unfavorably darker than CON and Lf-treated rabbits. Consumers regard bright cherry-red fresh meat color as an indicator of wholesomeness at the point of sale; deviations may influence purchase decisions (Mancini and Hunt 2005; Suman and Joseph 2013). Lactoferrin, an iron-containing glycoprotein, resulted in the lowest hue angle values and, thus, redder rabbit meat than the other groups. The red meat color obtained from a well-bled livestock carcass is mainly attributable to myoglobin (Mb) (Livingston and Brown 1981). In line with previous research, increasing the dietary Fe of broilers (Lin et al. 2020) and pigs (Apple et al. 2007) increased the redness value of meat color significantly. In this study, OXY-treated rabbits had higher meat redness and chroma values than controls but also had a higher hue value than Lf-treated rabbits, indicating less red meat (AMSA 2012). To better understand, the primary role of Lf in iron metabolism is regulating iron availability (Vorland 1999). Besides this, Lf oral administration tends to cause proteolytic degradation, which adversely affects iron binding/transferring property,

Table 8 Oxidative stress and antioxidant markers in liver and kidney tissues in rabbits of the different experimental TRTs

Item	CON	OXY	Lf	SEM	*P*-value
Liver					
MDA (nmol/ mg)	14.70[b]	26.92[a]	10.21[c]	0.600	0.0001
NO (µmol/ L)	22.17[b]	31.98[a]	12.29[c]	0.736	0.0001
CAT (µmH_2O_2/ Sec/ g tissue)	620.85[b]	598.23[c]	642.47[a]	2.398	0.0001
GST (U/ g tissue)	327.84[b]	300.48[c]	348.39[a]	2.003	0.0001
GPX (U/g tissue)	20.17[b]	14.34[c]	29.77[a]	0.658	0.0001
GSH (µmol/ g tissue)	103.67[b]	84.88[c]	123.39[a]	1.842	0.0001
Kidney					
MDA (nmol/ mg)	25.94[b]	42.12[a]	15.45[c]	0.672	0.0001
NO (µmol/ L)	32.48[b]	49.22[a]	25.18[c]	0.975	0.0001
CAT (µmH_2O_2/ Sec/ g tissue)	484.49[b]	458.78[c]	505.32[a]	2.042	0.0001
GST (U/ g tissue)	202.53[b]	185.46[c]	230.42[a]	1.536	0.0001
GPX (U/g tissue)	13.32[b]	8.90[c]	21.29[a]	0.830	0.0001
GSH (µmol/ g tissue)	84.88[b]	66.94[c]	95.20[a]	1.253	0.0001

[a], [b] Mean values in the same row with different superscripts differ significantly ($P<0.05$). GPX: Glutathione Peroxidase, GSH: Glutathione Reduced, GST: Glutathione-S-transferase, CAT: Catalase, NO: Nitric oxide

which is highly dependent on the structural integrity of Lf to bind its receptors (Wang et al. 2019).

Nonetheless, antioxidant availability, pH, and lipid oxidation are all important endogenous factors influencing meat color (Mancini and Hunt 2005). In the current study, Lactoferrin's antioxidant properties may also have prevented Mb from oxidation, causing better color stability (Joseph et al. 2012). Regarding shelf-life tests, which include APC and pH values, meat from Lf-treated rabbits outperformed meat from CON and OXY-treated rabbits. This may be directly linked to antimicrobial activity associated with intact Lf and peptides produced by its digestion, such as lactoferricin (Bellamy et al. 1992) and lactoferrampin (van der Kraan et al. 2005), which in some cases exhibits a more potent antibacterial activity. Direct application of Lf to beef subprimal cuts (Heller et al. 2007) and dietary supplementation to weaning pigs (Wang et al. 2007) both showed considerable antimicrobial activity against tested bacteria and pathogens such as *E. coli* and Salmonella. Lactoferrin's antimicrobial biological mechanisms include pathogen cell membrane rupture, virulence factor proteolysis, binding with glycosaminoglycans (GAGs) to inhibit microbial adhesion to host cells, and promoting beneficial microflora in the intestine (Ochoa and Cleary 2009; Superti et al. 2008; Superti and De Seta 2020).

The hematological parameters (RBCs, Hb, WBC, HCT, MCV, MCH, and MCHC) were increased in the Lf-treated rabbits and decreased in the OXY-treated rabbits compared with the CON group. The obtained results may be attributed to the immunomodulating effects of Lf through increasing the white blood cell counts and improving the hematological parameters (Costagliola et al. 2021; Poles et al. 2021). Abed et al. (2020) demonstrated that the immunomodulatory effects of Lf are emphasized by its effect on leukocyte composition and the virtual amounts of neutrophils and lymphocytes in the circulation. In addition to its positive effects on hematopoiesis, cytokine release, and cellular targets (Singh et al. 2023). Oral administered bLf has been evidenced for its role in elevating serum hematocrit and ferritin levels (Chierici et al. 1992). El-Sharawy et al. (2024) reported increased leucocytic count and Hb levels in Egyptian Baladi goats receiving Lf in 50, 100, or 200 mg/ day doses.

Regarding the impacts of Lf administration on the hepato-renal function, the results showed a reduction in the serum levels of AST, ALT, ALP, and urea in the Lf-treated rabbits and their increase in the OXY-treated rabbits compared with the CON group. The total, direct, and indirect bilirubin levels and creatinine and uric acid levels were increased in the OXY-treated rabbits. The total protein and globulin serum levels were increased in the Lf-treated rabbits and reduced in the OXY-treated rabbits. The albumin level decreased in the OXY-treated group. The reduced AST, ALT, and ALP levels indicate improved liver function. Likewise, a decrease in BUN was probably related to Lf's protective effect on kidney function. As well, Lf can also serve to prevent nephrotoxicity (Kimoto et al. 2013). These results were confirmed by the histological examination of the liver and intestinal tissues, which showed normal histoarchitecture of these tissues comparable to the CON rabbits.

In contrast, OXY-treated rabbits displayed minor morphological abnormality represented by focal hepatocellular hydropic degeneration, mild vascular dilatation, and biliary proliferation. These results indicated that Lf had no harmful effects on the tissues of the animals. El-Sherbeny and El-Shenawy (2023) reported impaired liver and kidney functions in the infected control rabbits indicated by a decrease in TP, albumin, globulin, and lysozyme activity and an increase in serum activities of (ALT AST, and ALP), BUN, and creatinine than the control group. All treated groups (Lf and antibiotic TRTs) recovered most of these parameters and improved general liver and kidney functions through a significant decrease in protein loss, tissue damage, and enzyme liberation expressed by a significant rise in lysozyme activity, TP, albumin, globulin, and Ca levels, and decrease in ALT AST, ALP, BUN and creatinine levels than the infected control group. Lactoferrin (500 mg kg^{-1} diet; 3 weeks) was demonstrated to have no significant effects on the serum levels of TP and BUN and AST activities in Japanese eel (*Anguilla japonica*) (Ren et al. 2007). Moradian et al. (2018) showed that Lf supplementation (200–800 mg

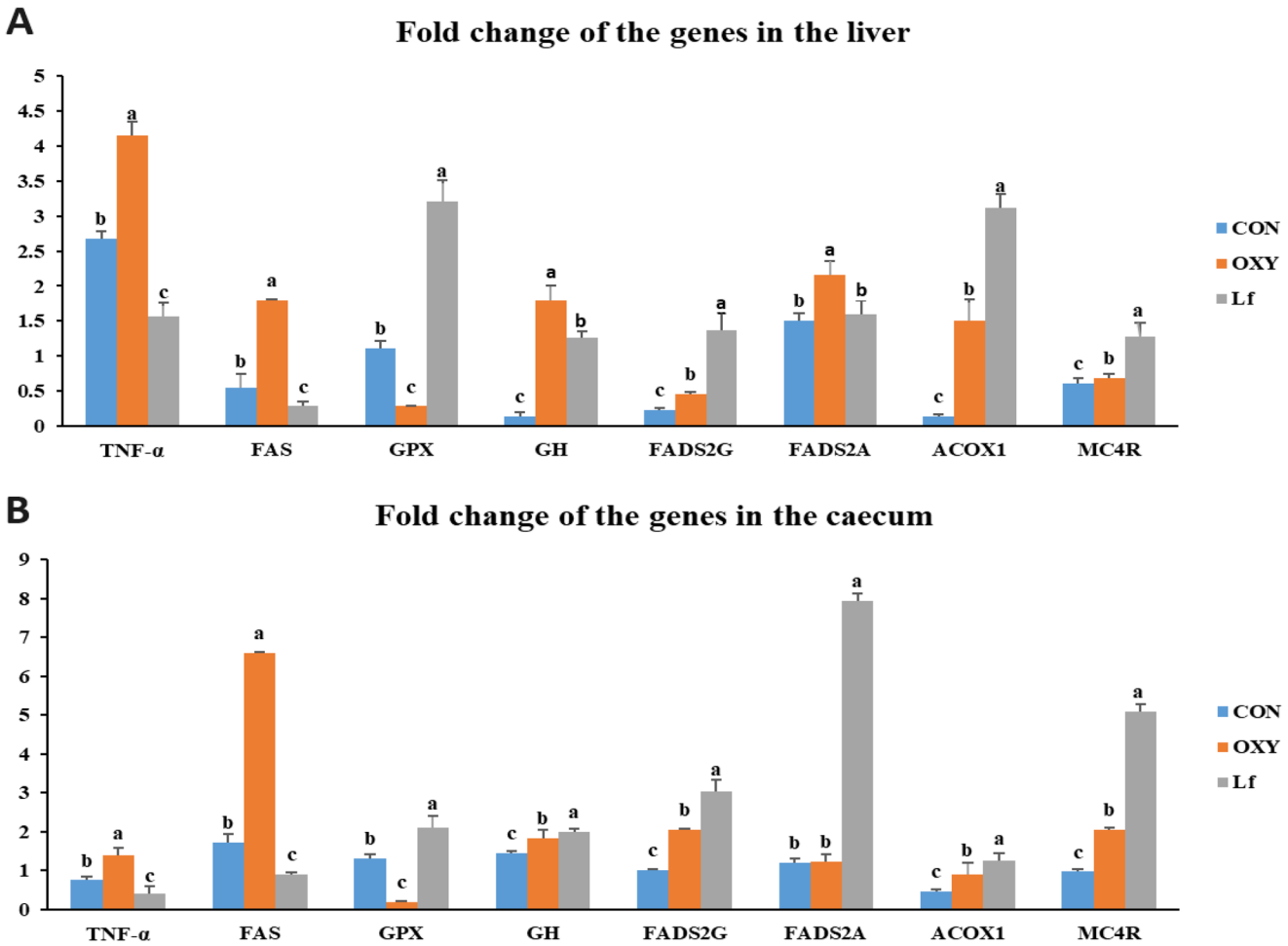

Fig. 5 Impact of the different TRTs on the genes related to growth, immunity, and antioxidant status in the liver (**A**) and caecum (**B**) of rabbits. a, b, c, d Mean values with different letters differ significantly ($P < 0.05$)

kg^{-1} diet) can reduce serum ALT and ALP activities. This indicates that Lf can improve liver function without affecting TP, albumin, or globulin levels.

The cytoprotective role of exogenous Lf against induced liver injury was recorded by (Guo et al. 2020) and (Fan et al. 2021), where Lf suppressed (hepatocellular death, inflammatory responses (TNF-α, IL-6, and nitric oxide) and endoplasmic reticulum stress), sustained the liver oxidative steadiness using nonenzymatic antioxidant manner, backed-up damaged hepatocytes autophagy, decreased ALT, AST, ALP, and γ-GT besides recovered hepatocyte siderosis through focusing on the hepcidin-ferroportin axis. Hepcidin acts as an antibacterial peptide formed in the liver and transferred all over the body, passing through the blood circulation to regulate the iron output of both hepatocytes and intestinal epithelial cells. Hence, all these belongings helped to restore regular liver function. Lf is classified as a "generally recognized as safe" (GRAS) substance, and many tests in animals have verified its safety and permissibility, even at increased doses (FDA 2014). (Flores-Villaseñor et al. 2012) assessed that Lf and its peptide effectively reduced sepsis hepatic and renal damage caused by infection and confirmed these findings histologically besides bacterial count where bacteria were not found in the kidney or liver after 72 h. Similar studies (Hsu et al. 2020; Zahan et al. 2022) examined the renoprotective effect of Lf against induced renal tubular damage in acute or chronic kidney diseases. They found that Lf reduced elevated BUN and creatinine levels and protected renal tissue histologically. Authors attributed these positive renal impacts to its functional bioactivities in maintaining the antioxidant enzyme activities, scavenging radicals, and augmenting autophagy, besides its antiproliferative and anti-inflammatory effects.

Concerning the effect of Lf administration on the immune status of rabbits, there was an increase in the serum levels of IgG and IgM in the Lf-treated animals compared to the CON and OXY-treated groups. The serum levels of IgA, IdD, and IgE were boosted in both Lf- and OXY-treated groups compared with the CON group. In addition, the expression of *TNF-α* and *FAS* genes was upregulated in

the OXY-treated group and downregulated in the Lf-treated group. FAS are efficient mediators of apoptosis (Nagata and Golstein 1995). Lactoferrin is an essential element of innate immunity (Wakabayashi et al. 2006). Lactoferrin levels are increased during infection or inflammation (Caccavo et al. 2003), indicating that Lf is an inflammation biomarker. Lactoferrin suppresses inflammatory reactions by regulating the production of inflammatory cytokines and interacting with macrophages (Crouch et al. 1992; Yamaguchi et al. 2001).

High affinity of Lf to iron makes it a valuable component in the nonspecific immune system (Baynes and Bezwoda 1994). It has been demonstrated that Lf can modify leukocytes, implicated in the innate immunity, by increasing the activity of the natural killer (NK) cells (Shau et al. 1992). Oral administration of bLf enhanced the host immune system by boosting the production of NK cells, CD4 + cells, and CD8 + cells in intestinal mucosa, interferon-γ (IFN-γ), and IL-10 in intestinal mesenteric lymph node cells and intraepithelial lymphocytes, interleukin-18 (IL-18) in intestinal epithelial cells of mice (Kanwar et al. 2015). Oral administration of bLF (0.1–2.5 g kg^{-1}) had positive effects on digestive and non-digestive tract infections in many animal models by activating the transcription of central immune-related genes in the small intestine and so encouraging systemic host immunity (Yamauchi et al. 2006). It is vital to host physiological activities with multipharmacological properties (Mayeur et al. 2016). It has anti-inflammatory, antimicrobial, and immunomodulatory characteristics, and all these activities can be reliant on or unrelated to its iron-binding capability. Its immunoregulatory effect is dependent on the actual host's immune status. Hence, it may activate immune cells to secrete specific cytokines that increase its anti-infectious activity and, in analogous, induce others that lower immune cells' excessive reactivity (Sienkiewicz et al. 2022) and also restrict inflammatory processes in septic inflammations. Lactoferrin supplementation is a beneficial method to improve antioxidant levels, stabilize the immune response, and normalize proinflammatory cytokines production, all of which are associated with intestinal health (Burrow et al. 2011a). Moradian et al. (2018) found that Lf increased lysozyme and bactericidal activities than the normal group. Authors suggested that connecting to immune cell receptors, supporting leukocytes function by positively enhancing natural killer cell, neutrophils, macrophages, and their phagocytic activities, bonding to bacterial cell walls, and limiting their intracellular proliferation are all means of immunomodulating properties.

Oxidative stress triggers the occurrence of some chronic degenerative conditions such as inflammation, neurodegenerative diseases, and cancer (Marnett 2000; Misonou et al. 2000). Certain pathophysiological conditions initiate reactive oxygen species (ROS) formation, which raises oxidative stress. In normal physiological conditions, various antioxidant enzymes, for instance, GPX, GST, and CAT, control the balance between ROS formation and their elimination (Kruzel et al. 2017). Our study exhibited that the hepatic and renal MDA and NO concentrations were decreased in the Lf-treated group and increased in the OXY-treated group. In contrast, the serum activity of CAT, GSH, GPX, and GST was improved in the Lf-treated group and decreased in the OXY-treated group. Also, the expression of the *GPX* gene was upregulated in the Lf TRT and downregulated in the OXY treatment. These results can be attributed to the significant and beneficial roles of Lf as it is considered a vital host defense compound and has been involved in many physiological functions like antioxidant activity, immunomodulatory activity, and antimicrobial/antiviral activities (Burrow et al. 2011b; Wakabayashi et al. 2006). The most important biological function of lactoferrin is iron chelation, which is involved in antimicrobial activity and antioxidant defense (Legrand 2016). Inflamed tissues produce high amounts of ROS, resulting in tissue necrosis and apoptosis. Moreover, damaged tissues discharge ferrous and ferric iron that participate in the Haber-Weiss reaction and produces free radicals. Lactoferrin's iron-scavenging ability significantly relieves oxidative stress (Fillebeen et al. 2001). Endogenic Lf is assumed to inhibit lipid peroxidation by iron impounding (Kanwar et al. 2015). Lactoferrin is an effective iron chelator that can decrease hydroxyl radicals and prevent cell oxidative damage (Sandomirsky et al. 2003).

Conclusion

From the results above, we can conclude that lactoferrin (300 mg /kg BW) is a promising growth promotor, immunomodulator, and antioxidant supplement that can alternate antibiotic growth promotors for rabbit production. Lactoferrin administration increased the rabbit weights by increasing the expression of growth-related genes. Furthermore, Lf improved the meat quality of rabbits by enhancing the color characteristics and prolonged the shelf life of meats. Lf improved the rabbit's health by improving blood hematology, maintaining good liver and kidney functions, and keeping the integrity of intestinal and liver tissues.

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Author contributions Conceptualization: TAI, IIS, EHH; methodology: TAI, IIS, EHH, SAA, SNE, LME, AE, EE, MHAMS, AO; formal analysis and investigation: AAK, ANA, SAA; writing—original draft preparation: SAA, AO, TAI; writing—review and editing: SAA, AO, TAI, EMY, A-WAA-W, SJD. All the authors approved the final draft.

Data availability Data will be available on reasonable request.

Declarations

Ethical approval The ethical approval of the experimental protocol was obtained from the Institutional Animal Care and Use Committee of Zagazig University, Egypt (Approval No: ZU-IACUC/2/F/101/2024). All animal experiments were performed based on the recommendations described in "The Guide for the Care and Use of Laboratory Animals in scientific investigations". All methods in the study were carried out in accordance with relevant institutional guidelines, and all animal experiments were performed following the ARRIVE guidelines.

Consent to participate Not applicable.

Consent to publish Not applicable.

Competing interests The authors declare that they have no conflict of interests. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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