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Specific hepatic gene responses to dietary fat levels during the finisher phase in a slow-growing chicken breed

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ABSTRACT

This study investigated the hepatic expression of genes involved in stress response (CASP6, CAT, SOD1, HSPA2) and lipid metabolism (LPL, SREBF, FABP1, ACOX1, FADS2) in a local slow-growing chicken breed, the Bionda Piemontese (BP), following two isonitrogenous diets (crude protein, 170 g/kg as fed): a control diet (L) and a high-fat diet (H) obtained by supplementing 6 % palm kernel oil, during the finisher period. Sixty (male and female) chickens were included in the study and slaughtered at five months of age. RNA was extracted from 36 liver samples (18 male and 18 female) and sequenced using the targeted RNA-seq method followed by bioinformatic analysis using DESeq2 to detect differences in gene expression. Overall, the high-fat dietary supplementation did not significantly alter the expression of most stress-related genes, indicating that the high-fat diet did not elicit a hepatic stress response in BP chickens. However, within each sex, the high-fat diet tended to upregulate *FADS2* and *FABP1* in females, and slightly downregulate *ACOX1* in males. Considering sex as an independent factor, *FABP1* expression was higher in females, whereas males exhibited significantly higher *LPL* expression. These findings highlight a clear sexual dimorphism in hepatic lipid gene expression in BP chickens. While dietary fat supplementation had limited impact on differentially expressed genes, the study underscores the importance of sex in shaping metabolic gene expression. It also provides evidence supporting the possible metabolic resilience of the BP breed to tolerate changes in dietary fat content. Further studies are needed to substantiate this claim, and to investigate the long-term effects and the tissue-specific responses of dietary fat supplementation in other organs.

Introduction

Local chicken breeds are increasingly valued for their contributions to sustainable agriculture, genetic conservation, and local resources (Fiorilla et al., 2024). Their role in sustainable agriculture is closely tied to the extensive farming system in which they are typically raised – namely, those characterized by low animal density and pasture – which promote animal welfare and reduce environmental impact (Rothrock Jr. et al., 2019). The genetic diversity preserved in these breeds not only enhances their adaptability to diverse environmental conditions but also serves as a valuable reservoir of traits that may be crucial for future breeding and sustainability efforts. The widespread reliance on commercial hybrids has contributed to a progressive loss of poultry biodiversity, resulting in the abandonment of local breeds, including the Bionda Piemontese (Castillo et al., 2021).

Bionda Piemontese (BP) is an Italian autochthonous chicken breed, mainly reared in the Piedmont region (Northwest Italy). It is a dual-purpose chicken, but at present it is mainly reared for meat production (Fiorilla et al., 2023). Its commercial size is approximately 1.8 kg for roosters and 1.3 kg for hens, and slaughtering is performed at about five months of age (Bongiorno et al., 2022), making it a 'slow-growing' breed (Soglia et al., 2020). For this reason, the productivity traits of this local breed are of critical relevance. However, local poultry productions are appreciated for their superior meat quality, reflecting their unique traits as well as their higher levels of welfare obtained through the extensive farming systems generally applied for such breeds (Marchewka et al., 2023; Afrin et al., 2024). These aspects, in turn, facilitate the potential for profitable productions from such breeds (Afrin et al., 2024). However, the high meat quality combined with slow growth performance also result in higher production costs, making the

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meat more expensive and thus less accessible to all consumers. The use of local chicken breeds for meat production requires conciliating economic growth with the benefits arising from the preservation of genetic biodiversity. Breeds like the BP play a crucial dual role in supporting local agricultural systems while contributing to biodiversity conservation.

Implementing dietary interventions during the finisher period may help mitigate costs while enhancing the production. In particular, reducing protein content and increasing energy levels has been shown to lower feed costs and enhance production performance (El-Deek et al., 2020). The use of supplemental fats is common practice to increase dietary energy density in poultry nutrition during the finisher period (Skřivan et al., 2018).

When evaluating the effect of an experimental diet, animal sex is a critical factor as it significantly influences metabolic responses. Male and female chickens may exhibit differences in intestinal structure and function, which can affect how nutrients are absorbed and utilized, ultimately impacting growth and overall performance (England et al., 2024).

Valuable insights into the underlying mechanisms of how diet and sex interact to influence physiological and productive responses can be obtained through the investigation of hepatic gene expression. Indeed, the liver plays a central role in regulating multiple metabolic pathways in chickens. It is the main site of lipid digestion, lipid transport, and fatty acid synthesis, all of which are essential for responding to dietary changes (Li et al., 2017; Yang et al., 2018; Guo et al., 2025). Changes in dietary fatty acid composition influence several enzymes involved in hepatic lipid metabolism, including lipoprotein lipase (*LPL*) (Goldberg et al., 2008), sterol regulatory element binding transcription factor 2 (*SREBF2*) (Goldberg et al., 2008), fatty acid-binding proteins (*FABP*) (Murai et al., 2009), fatty acid desaturase 2 (*FADS2*), and acyl-coenzyme A oxidase 1 (*ACOX1*) (Li et al., 2017). Moreover, the liver plays a crucial role in the stress response, as it expresses the main antioxidant enzymes such as caspase (*CASP*), catalase (*CAT*), superoxide dismutase (*SOD*), and heat-shock protein (*HSP*) in response to stressful conditions (Rimoldi et al., 2015). Notably, hormonal differences between the two sexes may condition the liver's response to stress and lipid metabolism, which can influence its function. Evaluating the expression of candidate genes involved in the hepatic stress response and lipid metabolism can provide useful information about the separate and combined effects of diet and sex on poultry performance, helping us understand the impact (and tolerability) of a high lipid diet in a specific breed (Desert et al., 2018).

The aim of this study was to determine the modulation of hepatic gene transcription related to stress and lipid metabolism in both male and female chickens of a slow-growing local breed to improve the understanding of the impact of dietary fat levels during the finisher period. Specifically, the study evaluated how different levels of dietary fat inclusion and sex influenced the expression of genes involved in lipid metabolism and stress response in the liver and their effect on growth traits, carcass performance, and meat quality. Understanding the interaction between diet and sex on hepatic gene expression is essential for optimizing poultry production systems – particularly in local chicken breeds – as it provides insights into their dietary adaptability. Such analyses can support the development of nutritional strategies that enhance overall performance without compromising meat quality, while accounting for sex-specific physiological responses.

Materials and methods

The experimental protocol was developed in compliance with the current European Directive (2010/63/EU) concerning the care and use of animals for scientific purposes, and received approval from the Bioethical Committee of the University of Turin (Prot. ID:251833).

Experimental design

The study was conducted at the poultry facility of the Department of Veterinary Sciences of University of Turin (Italy) on 60 BP chickens, 30 from each sex, randomly divided into 6 pens (5 birds/pen). Until 120 days of age, all chickens were reared under a free-range farming system; the birds were exposed to natural temperature conditions and photoperiod, with unrestricted access to open areas. All birds were fed the same starter and growing diet. During the finisher period (120-150 days of age), chickens of both sexes were divided in two groups: one was fed a standard diet (control diet: low-fat, L) and one was fed the standard diet supplemented with 6 % palm kernel oil (experimental diet: high-fat, H). The two diets contained the same percentage of crude protein (17 %), but different ether extract content (EE), meaning that the two diets were isonitrogenous but not isoenergetic (Table 1).

Performance and meat quality parameters

The average daily feed intake per bird was calculated at the pen level every 15 days throughout the experimental period. All birds were individually weighed every two weeks. At the age of 150 days, all chickens were slaughtered, plucked, and eviscerated, and the weights of hot carcasses, organs, and abdominal fat content recorded. After 24 h, both carcass and meat quality parameters were evaluated. Measurements included carcass weight, ready-to-cook carcass weight (without head and legs), breast weight, and whole leg weight (thigh and drumstick). The meat quality parameters assessed were pH, color, thawing loss, cooking loss, and tenderness.

The pH and color parameters were measured on thigh and breast samples in duplicate using a SevenGO SG2^{TM} pH-meter (Mettler-Toledo, Schwerzenbach, Switzerland) and a Minolta CR-200 Chroma Meter (Konica Minolta, Chiyoda, Japan), respectively. The color parameters, L^* (lightness), a^* (redness index), and b^* (yellowness index), were measured according to the CIELab system (Commission Internationale de l'Éclairage, 1976).

The thigh and breast samples were then frozen at −20°C and thawing loss subsequently calculated as the difference in weight of meat before (W_{bt}) and after (W_{at}) thawing, and expressed as the percentage of the

Table 1
Ingredients (g/kg as fed) and formulated chemical composition of the control (low-fat; L) and experimental (high-fat; H) diets during the finisher period.

Ingredients	L	H
Corn meal	700	625
Soybean meal	224	239
Palm kernel oil	–	60
Sunflower seed oil	40	40
Calcium carbonate	12	12
Dicalcium phosphate	10	10
Vitamin-mineral premix[1]	10	10
Soybean oil	5	5
Cane molasses	5	5
Sodium chloride	2	2
Sodium bicarbonate	1	1
Metabolizable energy (kcal/kg)	2983	3264
Chemical composition (formulated)		
Dry matter (%)	88.66	86.51
Crude protein (%)	17.00	17.00
Ash (%)	4.81	4.80
Ether extract (%)	3.85	9.49
Crude fiber (%)	4.06	3.99

Abbreviations: L: low-fat; H: high-fat; DM: dry matter.
[1] Premix contained the following nutrients (units are expressed per kg of diet): vitamin A, 15,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B_1, 2 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; vitamin B12, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

initial sample weight:

$$\text{Thawing loss (\%)} = [(W_{bt} - W_{at})/(W_{bt})] \times 100$$

Cooking loss and shear force were analyzed at the Department of Agriculture, Food, Environment, and Forestry (DAGRI, University of Florence, Italy). These parameters were only assessed for breast meat samples. For the determination of breast cooking loss, about 70 g of breast muscle was vacuum-sealed and cooked in a hot water bath at 63°C for 1 h (SV2447, Severin, Sundern, Germany). The weight difference before (W_b) and after (W_a) cooking was calculated according to Equation:

$$\text{Cooking loss (\%)} = [(W_b - W_a)/(W_b)] \times 100$$

Color measurements were also conducted after cooking, specifically on the external surface. Tenderness was measured using shear force measurements, which were calculated as an internal force generated by an applied load on the meat portion. Shear force was measured on both raw and cooked meat proportion (3 × 3 × 1 cm) obtained from the center-right and center of the breast sample, respectively. The texture analyzer (Z1.0, Zwick/Roell, Ulm, Germany) was set with a loading cell of 100 N and a crosshead speed of 250 mm/min, according Haghighi et al. (2021). Data collection was performed by testXpert® II V3.0 software (Zwick/Roell, Ulm, Germany).

Tissue collection and RNA extraction

On the date of slaughter (150 days), thirty-six liver samples (9 samples/diet/sex) were collected in RNA later and then stored at −80°C until extraction. Total RNA was extracted with the NucleoSpin® RNA Plus Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). RNA was assessed using the Qubit® RNA Broad-Range Assay Kit, while integrity (RIN) was evaluated with Agilent 2100 Bioanalyzer.

Multiplex digital expression gene analysis (MuDEGA)

Nine genes were identified from the literature as candidate genes linked to stress and lipid metabolism: catalase (*CAT*), caspase 6 (*CASP6*), fatty acid desaturase 2 (*FADS2*), acyl-CoA oxidase 1 (*ACOX1*), lipoprotein lipase (*LPL*), sterol regulatory element binding transcription factor 2 (*SREBF2*), heat shock protein 70 (*HSP70*), superoxide dismutase (*SOD1*), and fatty acid binding protein 1 (*FABP1*) (Rimoldi et al., 2015; Li et al., 2017; Srikanthithasan et al., 2024). Actin beta (*ACTB*) and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were used as housekeeping genes, as suggested by ReFinder software and the BestKeeper program (Pfaffl et al., 2004). The primer sequences are listed in in Table 2.

Multiplex digital expression gene analysis (MuDEGA) was performed by next generation sequencing (NGS) using a Miseq™ (Illumina®, San Diego, USA) instrument, as descripted by Raspa et al. (2023). In brief, three biological replicates for each group were reverse transcribed using the First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) and the resulting cDNA was quantified using the Qubit® DNA High-Sensitivity Kit. For NGS sequencing, the library was prepared by purifying the multiplex PCR products (ExoSAP-IT® Express), followed by index PCR (Nextera® XT Index kit, Illumina inc., San Diego, USA). The products of the index PCR reaction were quantified, and their sizes analyzed using an Agilent 2100 Bioanalyzer. Each library was diluted to 4 nM and pooled for NGS sequencing on the Miseq Illumina platform. The targeted RNAseq data (primary and secondary analysis) were analyzed using Local Run Manager software (LRM, Illumina®, San Diego, USA), and mapped using the BWA-MEM algorithm in LRM. Paired-end qualified reads were mapped to the Custom References Genes panel using the NCBI reference gene sequences (Table 2, Accession num.). The resulting BAM files were exported and used for bioinformatic analysis; one gene was counted for each aligned read, having only one amplicon per mRNA.

Bioinformatic analysis

Statistical analyses for performance and meat quality parameters were performed in R software (version 4.2.2). The assumption of normality and homogeneity of variance was assessed using the Shapiro-Wilk and Levene's test, respectively. Two-way ANOVA and the Kruskal Wallis test were performed for normally and non-normally distributed data, respectively. Post-hoc tests (Tukey-Kramer and Dunn tests for normally and non-normally distributed data, respectively) were used to identify specific differences among groups.

The FeatureCounts package was used for counting reads (Liao et al., 2014), and DESeq2 package for differential expression analysis. Gene expression was normalized using the median ratio method in DESeq2 R package (Love et al., 2014) with housekeeping genes specified for size factor estimation. A different gene expression was considered for p-adjusted value < 0.05, and a log2 fold-change (log2FC) > 1.0 and < -1.0. The p-value adjustment was made using the Benjamini and

Table 2
Oligonucleotide primer pairs used for multiplex digital expression gene analysis (MuDEGA).

No	Gene	Primer Sequence (5′−3′)	Accession num.	Reference
1	CAT	F: ACTGGTGCTGGCAACCC R: ACGTGGCCCAACTGTCAT	NM_001031215	Rimoldi et al., 2015
2	CASP6	F: CGTTAATCTTCAATCACGAGCACTT R: GCGTTTCAGATTGTTTCTGTCTGC	NM_204726	Rimoldi et al., 2015
3	FADS2	F: ACTGGTGGAACCATCGTCAC R: GCAGAGGTGGGAAGATGAGG	NM_001160428	Li et al., 2017
4	ACOX1	F: CCAGTCAGCTTGGTAGAGGC R: AGTGACAGTGTGCCTCAGATG	NM_001006205	Li et al., 2017
5	HSP70	F: CCAAGAACCAAGTGGCAATGAA R: CATACTTGCGGCCGATGAGA	EU747335	Rimoldi et al., 2015
6	LPL	F: TTGGTGACCTGCTTATGCTA R: ATTGCTGCCTCTTCTCCTTT	NM_205282	Li et al., 2017
7	SREBF2	F: AGCCTCAGATCATCAAGACG R: TTCCATTGCTCCCAACAAGG	AJ310769	Li et al., 2017
8	SOD1	F: CGGGCCAGTAAAGGTTACTGGAA R: TGTTGTCTCCAAATTCATGCACATG	NM_205064	Rimoldi et al., 2015
9	FABP1	F: GAAGGGTAAGGACATCAA R: TCGGTCACGGATTTCAGC	NM_204192.3	Li et al., 2017
10	ACTB	F: AAATCAAGATCATTGCCCCACCT R: AGGGGTGTGGGTGTTGGTAA	NM_205518	Li et al., 2017
11	GAPDH	F: GCTAAGGCTGTGGGGAAAGT R: TCAGCAGCAGCCTTCACTAC	NM_204305	Li et al., 2017

Hochberg's approach for controlling the false discovery rate. Gene expression differences in the normalized read counts data set were explored by Principal Component Analysis (PCA) using the variance stabilizing transformation function (vst) (Anders and Huber, 2010). Differential gene expression was assessed by comparing all groups (HF, HM, LF, LM) in a pairwise fashion. The effect of sex was evaluated independently of diet, whereas the effect of diet was assessed both within each sex and across the entire dataset. Visualization of contrasts between sex and diet groups were performed using the Enhanced Volcano package in R (Blighe et al., 2018).

Results

Performance and meat quality parameters

Performance parameters

The effects of diet and sex on body weight and daily feed intake during the experimental period are presented in Table 3. No significant differences were observed across diets throughout the experiment. However, differences were noted between sexes. Males had a significant higher body weight and daily feed intake compared with females during the finisher period ($P < 0.001$).

Carcass parameters

Carcass parameters include the weight of the whole carcass, eviscerated carcass, carcass without head and legs (ready-to-cook), breasts, thighs and drumsticks, and abdominal fat content. Table 4 shows the carcass performances of BP chickens. The experimental diet had no significant effect on any of the carcass parameters. By contrast, it was observed a highly significant ($P < 0.001$) effect of sex on all the carcass traits evaluated.

Meat quality parameters

The mean values of the meat's physical traits (pH, color, thawing loss, cooking loss, and shear force) are shown in Table 5. The experimental diet had a significant effect on the thawing loss percentage in breast meat samples. Specifically, male chickens fed the H diet exhibited less thawing loss compared with those fed the L diet ($P < 0.05$). Sex also had a significant effect on meat physical traits, except for pH, thawing loss, cooking loss in breast meat, and the a^* value in thigh samples (Table 5).

Table 3
Effect of dietary treatment and sex on the performance parameters[1] of BP chickens at 120, 135, and 150 days of age.

Group	Body weight (g)			Daily feed intake/bird (g)		
	120 days	135 days	150 days	120 days	135 days	150 days
LM	2,610	2,820	2,870	139	147	149
HM	2,650	2,870	2,910	152	146	142
LF	1,760	1,940	2,000	106	102	103
HF	1,740	1,940	2,000	100	103	99
SEM[2]	62	64	63	6	6	6
Main effect						
Diet						
L	2,180	2,380	2,440	122	124	126
H	2,260	2,470	2,520	130	127	124
SEM[2]	88	90	90	9	9	9
Sex						
Male	2,630	2,840	2,890	145	146	145
Female	1,750	1,940	2,000	103	102	101
SEM[2]	30	32	34	4	3	4
P-value						
Diet	0.10	0.07	0.12	0.20	0.63	0.74
Sex	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Diet × Sex	0.53	0.61	0.59	0.12	0.81	0.81

Abbreviations: L: low-fat; H: high-fat; M: male; F: female.
[1] Data are mean values (rounded to the first significant figure).
[2] SEM: standard error of the mean.
$^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

Multiplex digital expression gene analysis results

The average mapping rate across all samples was 92 %. The average base quality score exceeded Q30 in approximately 94 % of the reads per sample. Following quality control, all 36 samples were included in subsequent analyses. The PCA plot (Fig. 1) showed no differences between experimental groups, according to the comparison of principal components 1 and 2 (PC1 and PC2). Additionally, a high inter-individual variability was observed, as illustrated in the plot of normalized gene counts (Fig. 1).

Diet effect on liver gene expression

To evaluate the possible effect of the change in diet during the finisher period on hepatic gene expression, the experimental diet group was compared with the control diet group (males and females combined) as well as for the two sexes separately. *ACTB* was excluded because its expression levels varied significantly between the different experimental groups, indicating a lack of stability and reliability for normalization purposes. Fig. 2A shows the volcano plots resulting from the comparison of the two diets. Although no genes were differentially expressed in response to the change in dietary treatments, the log2FC analysis indicates a trend toward upregulation for *FABP1* gene in chickens fed the H diet (p-adjusted value= 0.06; log2FC= 1.14) (Fig. 3). When males and females were analyzed separately, again no genes were found to be differentially expressed in either males (LM vs HM) or females (LF vs HF) (Fig. 2B and 2C). However, it was observed a consistent trend of upregulation for *FABP1* and *FADS2* (log2FC= 1.52 and 1.65, respectively) in females fed the H diet, although the differences did not achieve statistical significance (p-adjusted value= 0.17 and 0.07, respectively) (Fig. 3). In males, a modest but significant downregulation of *ACOX1* was observed in chickens fed the H diet (p-adjusted value= 0.04; log2FC= −0.74) (Fig. 3).

Sex effect on liver gene expression

The overall effect of sex on the expression of hepatic genes linked to stress and lipid metabolism is represented as a volcano plot in Fig. 2D. We did not assess the effect of sex within the two dietary groups because i) no significant effect of diet or strong trend was detected, and ii) combining all samples from both sexes allowed us to maximize sample size and obtain more reliable results. The male group was taken as the reference group for comparative analysis; hence, the up- and down-regulation of genes refer to the M group with respect to the F group. Lipoprotein lipase (*LPL*) was upregulated in male chickens (p-adjusted value < 0.05; log2FC= 1.85), and fatty acid binding protein 1 (*FABP1*) was downregulated in male chickens (p-adjusted value < 0.05; log2FC= −1.05). The difference in *HSPA2* expression between males and females is significant, although the fold change is relatively low (log2FC= 0.80).

Discussion

The aim of this study was to evaluate the hepatic transcription of candidate genes involved in the hepatic stress response (*CASP6, CAT, SOD1*, and *HSPA2*) and lipid metabolism (*LPL, SREBF, FABP1, ACOX1*, and *FADS2*) to test the adaptability of a local slow-growing chicken breed – the Bionda Piemontese – to dietary fat supplementation during the finisher period.

In general, the experimental diet did not affect carcass traits or the meat quality of either male or female chickens, with just a few exceptions. The lack of any significant differences in most parameters suggests that both diets are generally suitable for maintaining productive performance and meat quality in BP chickens, regardless of sex. This is a desirable trait for poultry production systems, as it allows for a certain flexibility in the diet formulation without compromising overall

Table 4
Effect of dietary treatment and sex on Bionda Piemontese carcass parameters[1].

Group	Carcass (g)	Eviscerated carcass (g)	Ready-to-cook (g)	Thighs and Drumsticks (g)	Breasts (g)	Abdominal fat (g)
LM	2,340	2,150	1,840	680	289	16
HM	2,300	2,090	1,800	650	281	35
LF	1,630	1,420	1,280	410	232	59
HF	1,600	1,390	1,240	400	217	67
SEM[2]	50	50	40	18	5	5
Main effect						
Diet						
L	1,980	1,790	1,560	549	260	37
H	2,000	1,790	1,560	545	253	49
SEM[2]	71	71	57	26	1	6
Sex						
Male	2,320	2,120	1,820	670	285	25
Female	1,620	1,410	1,260	407	225	62
SEM[2]	274	24	23	9	4	5
P-value						
Diet	0.63	0.99	0.97	0.75	0.40	0.13
Sex	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Diet × Sex	0.76	0.73	0.97	0.62	0.54	0.49

Abbreviations: L: low-fat; H: high-fat; *M*=male; *F*=Female.
[1] Data are mean values (rounded to the first significant figure).
[2] SEM: standard error of the mean.

Table 5
Effects of dietary treatment and sex on Bionda Piemontese breast and thigh meat quality parameters[1].

Group	pH	a*	b*	L*	Thawing loss (%)	Cooking loss (%)	Shear force Raw meat (N/g)	Shear force Cooked meat (N/g)	a* cooked	b* cooked	L* cooked
Breast											
LM	5.71	3.07	−0.30	53.85	6.50[a]	7.90	6.20	2.50	3.50	11.50	80.40
HM	5.72	2.90	0.80	54.03	4.30[b]	8.30	6.60	2.40	3.30	11.80	80.80
LF	5.63	1.70	5.60	52.70	5.50[a,b]	8.80	4.50	2.00	2.10	13.00	82.50
HF	5.65	1.50	5.60	53.06	5.50[a,b]	9.00	5.00	2.00	1.90	12.60	83.30
SEM[2]	0.02	0.10	0.40	0.20	0.20	0.20	0.20	0.06	0.10	0.10	0.60
Main effect											
Diet											
L	5.76	2.40	2.70	53.30	6.00	8.30	5.30	2.23	2.80	12.30	81.50
H	5.69	2.30	2.90	53.60	4.80	8.60	5.90	2.28	2.70	12.10	81.90
SEM[2]	0.03	0.20	0.50	0.30	0.20	0.30	0.20	0.09	0.20	0.20	0.40
Sex											
Male	5.72	3.00	0.30	53.90	5.40	8.10	6.40	2.45	3.40	11.60	80.60
Female	5.64	1.60	5.60	52.80	5.50	8.90	4.70	2.00	2.00	12.90	82.90
SEM[2]	0.03	0.10	0.20	0.30	0.30	0.30	0.20	0.08	0.10	0.20	0.30
P-value											
Diet	0.47	0.71	0.54	0.63	< 0.01	0.55	0.08	0.65	0.42	0.59	0.35
Sex	0.07	< 0.001	< 0.001	<0.01	0.51	0.06	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Diet × Sex	0.88	0.81	0.13	0.17	< 0.001	0.94	0.80	0.53	0.89	0.12	0.61
Thigh											
LM	5.90	15.90	10.50	45.00	0.45	-	-	-	-	-	-
HM	5.90	15.60	10.30	45.70	0.54	-	-	-	-	-	-
LF	6.00	15.40	11.40	46.20	0.63	-	-	-	-	-	-
HF	6.00	15.80	11.30	47.00	0.69	-	-	-	-	-	-
SEM[2]	0.02	0.10	0.10	0.20	0.03						
Mean effect											
Diet											
L	5.93	15.60	11.00	45.60	0.54	-	-	-	-	-	-
H	5.95	15.70	10.80	46.30	0.61	-	-	-	-	-	-
SEM[2]	0.02	0.20	0.20	0.30	0.03						
Sex											
Male	5.90[a]	15.70[a]	10.40[a]	45.35[a]	0.50	-	-	-	-	-	-
Female	6.00[b]	15.60[a]	11.35[b]	46.60[b]	0.65	-	-	-	-	-	-
SEM[2]	0.02	0.20	0.20	0.30	0.03						
P-value											
Diet	0.69	0.70	0.46	0.18	0.25	-	-	-	-	-	-
Sex	< 0.001	0.53	< 0.001	< 0.01	< 0.01	-	-	-	-	-	-
Diet × Sex	0.53	0.30	0.83	0.94	0.80	-	-	-	-	-	-

Abbreviations: L: low-fat; H: high-fat; *M*=male; *F*=Female.
[1] Data are mean values (rounded to the first significant figure).
[2] SEM: standard error of the mean.
[a,b] Values in the same column annotated with different superscript letters are significantly different ($P < 0.05$).

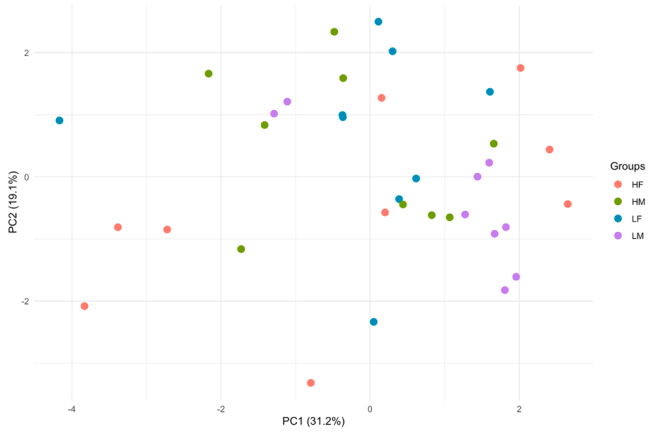

Fig. 1. Principal Component Analysis of normalized gene counts[1].
[1]PCA scatter plot of normalized gene counts, showing the first (PC1) and second (PC2) principal components. Samples are grouped according to diet (H: high-fat; L: low-fat) and sex (F: female; M: male).

performance or meat quality. One significant effect of the high-fat content diet, however, was a reduction in the thawing loss of breast meat from male birds, which may represent a positive outcome. A higher thawing loss implies greater moisture loss, potentially resulting in drier and less tender meat (Zhang and Ertbjerg, 2019), which could have been affected by the pH (Qiao et al., 2001; Šefcová et al., 2021; Yalçin et al., 2024), or be related to an effect on the myofibrillar protein structure (Leygonie et al., 2012; Yalçin et al., 2024). In our study, however, no significant differences in pH were observed between groups, suggesting that the reduction in thawing loss associated with the high-fat diet is more likely related to differences in water-holding capacity, potentially driven by metabolic changes or alterations in muscle structure. Most of the observed significant differences were primarily related to sex. The BP males were heavier than the females, in alignment with the data reported by Bongiorno et al. (2022), and exhibited lower abdominal fat deposition, and redder, tougher meat compared with females.

The effect of high-fat supplementation during the finisher period in BP chickens on hepatic transcription revealed no significant alterations in the expression of genes encoding the antioxidant enzymes *SOD* and *CAT*. Similarly, the lack of a change in *CASP6* expression suggests that fat supplementation, at the tested level, does not strongly activate apoptotic pathways in the liver. Additionally, the expression of *HSPA2*, a member of the heat shock protein 70 family, involved in protein folding and protection against proteotoxic stress, remained stable, further supporting the notion that the dietary intervention did not elicit a strong stress response at the molecular level. These genes are likely to exhibit more pronounced changes in expression under prolonged, strong or acute stress conditions, such as heat exposure, which is known to induce robust cellular stress responses in the liver (Rimoldi et al., 2015). However, a modest but significant difference in *HSPA2* expression was observed between sexes. This suggests that the difference may be intrinsically linked to sex-related physiological or hormonal factors. In fact, sex hormones (testosterone and estrogen), are known to influence the expression of stress-related genes and may modulate chaperone activity in a tissue-specific manner.

In the context of hepatic lipid metabolism, *FADS2* and *FABP1* showed a tendency to be upregulated in females fed the H diet. The slight upregulation of *FADS2*, a gene involved in the biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFAs), may indicate an adaptive response to the dietary fat sources, promoting the endogenous synthesis of such fatty acids. This result lies in accordance with the chemical composition of breast meat, with females fed the H diet showing non-significant trend towards higher levels of PUFAs compared with those fed the C diet (PUFAs = 13.4 and 12.9 mg/g dry matter, respectively; data not shown). The male group showed a similar content of PUFAs between diets, which aligns with the absence of any differential *FADS2* expression observed in this group.

Expression of the *FABP1* gene has already been shown to be associated with abdominal fat weight in chickens (Wang et al., 2019), with higher expressions linked to increased mitochondria fatty acid transport for oxidation, rather than for fat deposition (Wang et al., 2006). This suggests that the high lipid content in the diet was primarily utilized for β-oxidation. As a result, no significant differences in abdominal fat deposition were observed between the two diets in females.

In males, a significant, but modest downregulation of *ACOX1* was observed in chickens fed the H diet. Interestingly, higher expression levels of this gene have been reported in response to diets enriched with alanine (Li et al., 2017). In the present study, the fatty acid composition of the two diets was not evaluated; however, further analysis of the lipid profiles could help clarify the observed differences in gene expression.

Most of the significant differentially expressed genes were observed between males and females. Sex-dependent differences in liver gene expression linked to fatty acid metabolism in local poultry breeds have previously been identified, highlighting a sexual dimorphism (Perini et al., 2023). In our case, *FABP1* and *LPL* genes were differentially expressed between sexes. *FABP1* genes was upregulated in females. This finding aligns with a previous report indicating an abundant hepatic expression of *FABP1* in hens (Wang et al., 2019). Therefore, the observed differences in *FABP1* mRNA expression appear to be primarily driven by sex, suggesting a sex-related response. Conversely, males exhibited higher expression of the lipoprotein lipase (*LPL*) gene. This result could explain significant differences in lipid metabolism between the two sexes. The higher expression of *LPL* may enhance the utilization of fat-derived energy, supporting increased physical activity, a factor that may have contributed to the firmer meat texture observed in males compared with females. Similar results were reported by Cui et al. (2021) in a crossbreed (Turpan cockfighting × White Leghorn) showing that the high *LPL* expression is negatively correlated with fat deposition and positively associated with energy production in male chickens. Additionally, sex hormones, particularly estrogens, may play a key role in modulating fat accumulation in females, indirectly influencing the expression of genes involved in lipid metabolism, including the *LPL* gene (Zhao et al., 2024). Thus, the differential expression of *LPL* and its impact on lipid metabolism could help explain the observed variation in the shear force between male and female chickens. However, several limitations of the study must be considered. First, the analysis focused exclusively on hepatic gene expression, neglecting potential contributions from other metabolically relevant tissues, such as adipose tissue and skeletal muscle. Moreover, levels of active *LPL* protein or its enzymatic activity were not analyzed, which could provide further insights into functional differences between sexes.

Although high-fat dietary inclusion did not markedly alter most of the measured parameters, the study highlights the adaptability of BP chickens to different diets without compromising overall performance or meat quality. The results suggest that this breed may possess a certain level of metabolic 'robustness', enabling it to maintain performance under varying nutritional regimes. However, further research is needed to explore the long-term effects of dietary supplementation on BP chickens, particularly focusing on the interaction between sex, metabolism, and diet.

Conclusions

In conclusion, this study highlights the interactions between sex and diet on gene expression in Bionda Piemontese chickens, a slow-growing local breed, specifically in relation to liver metabolism. The results suggest that a high-fat dietary supplementation during the finisher period did not lead to broad effects on the metabolism of this local breed. However, sex emerged as a key factor influencing this parameter. Particularly, sex-related differences were observed in *LPL* gene

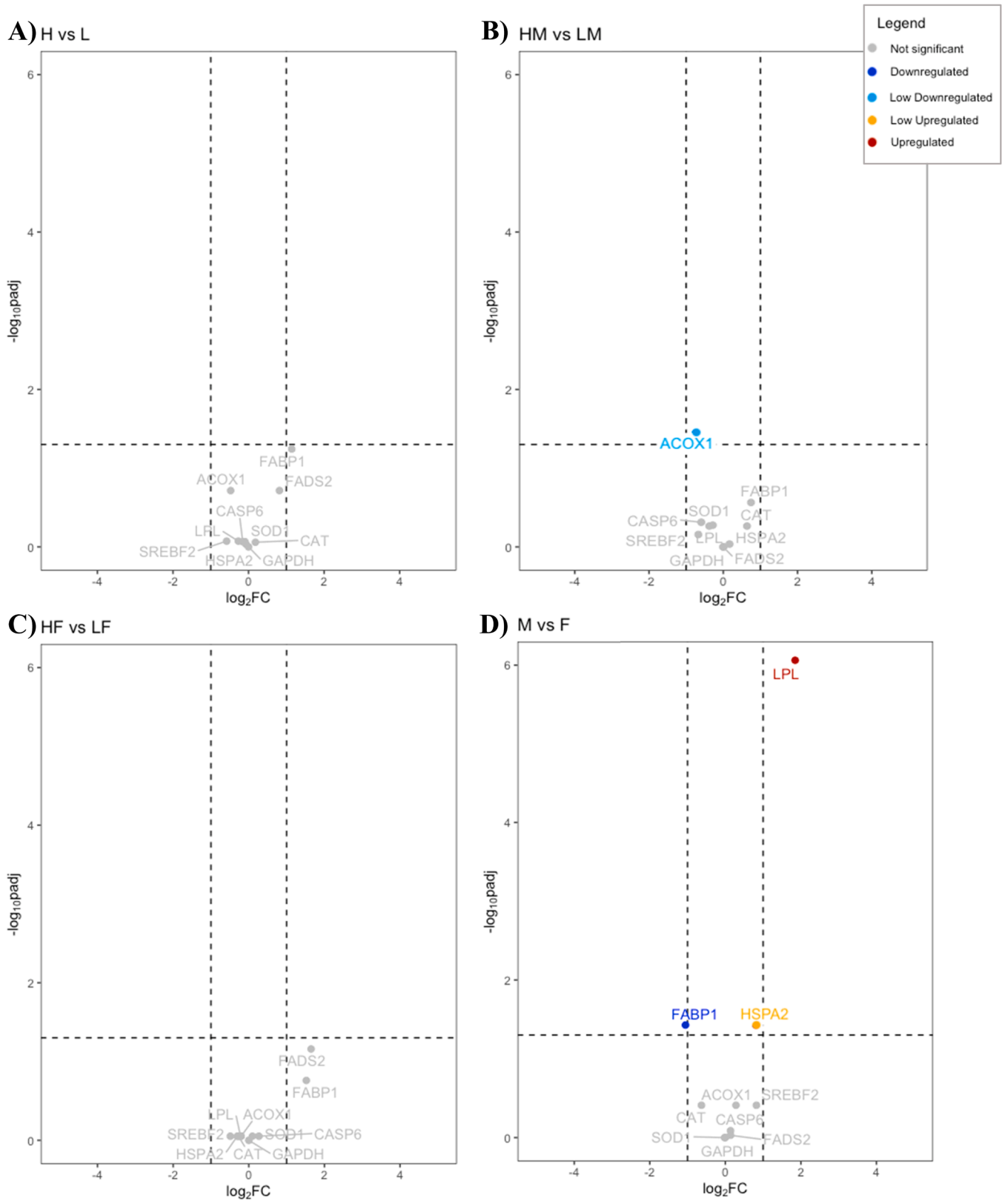

Fig. 2. Volcano plot of differentially expressed genes (DEGs)[1].

[1]Volcano plot of nine genes related to lipid and stress metabolisms. Grey dots represent genes below the level of significance (p-value > 0.05 and $-1.0 < log_2FC < 1.0$), blue dots represent significantly downregulated genes ($log_2FC < -1.0$; p-value < 0.05), light blue and orange dots represent significant but low FC ($-1.0 < log_2FC < 1.0$; p-value < 0.05), and red dots represent significantly upregulated genes ($log_2FC > 1.0$; p-value < 0.05). **A)** Shows the different gene expression between high-fat (H) and low-fat(L) diets, without considering the sex. **B)** Shows the different gene expression between diets (H and L) in male (M) group, while **C)** in female (F) group. **D)** Represents the different gene expression between sex (M and F).

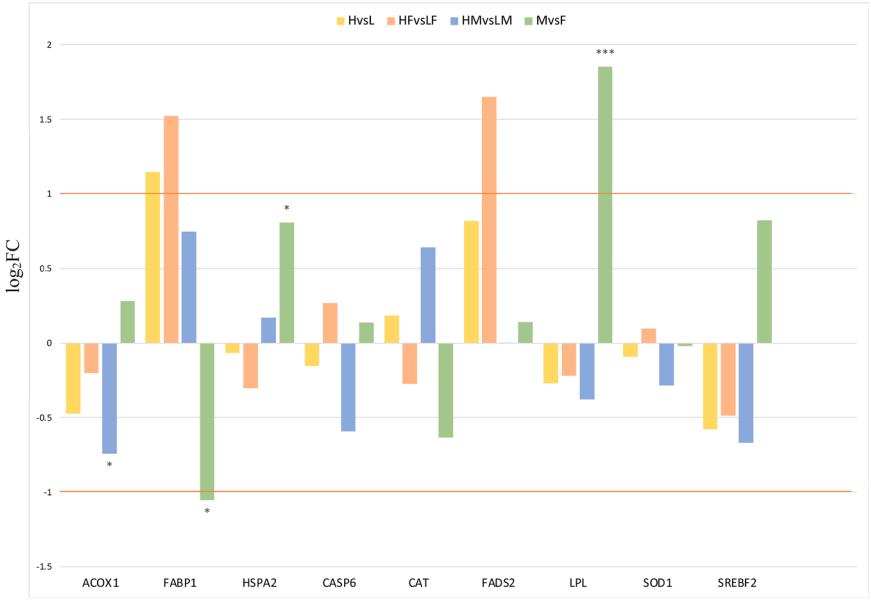

Fig. 3. Log2 Fold Changes (FC) in gene expression across experimental conditions[1].
[1]Log2 Fold Changes of stress metabolism genes (HSPA2, CASP6, CAT, SOD1) and lipid metabolism genes (ACOX1, FABP1, FADS2, LPL, SREBF2) expression levels between H (high-fat) and L (low-fat) diet in male (M) and female (F) chickens. The red lines indicate the log_2FC threshold used to define genes with a great alteration, while * indicate the significance of this alteration: * p-value < 0.05; *** p-value < 0.001.

expression, suggesting that these differences may help explain variations in lipid metabolism and body composition between males and females. Further research should explore the use of other lipid sources in local poultry production, emphasizing the interactions between genetic, hormonal, and environmental factors. Such studies should aim to elucidate the mechanisms driving these differences and their implications for metabolic health, ultimately working toward the development of alternative, cost-effective feed solutions.

CRediT authorship contribution statement

N. Stoppani: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **G. Secci:** Writing – review & editing, Methodology, Formal analysis. **F. Raspa:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **G. Parisi:** Writing – review & editing, Supervision, Formal analysis. **J. Nery:** Software, Investigation. **C. Bianchi:** Methodology, Investigation. **V. Zambotto:** Methodology, Investigation. **M. Profiti:** Methodology, Investigation. **E.E. Cappone:** Writing – review & editing, Investigation, Formal analysis. **A. Schiavone:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **D. Soglia:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Disclosures

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dominga Soglia reports financial support was provided by Piedmont Region. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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