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Effect of Algae Supplementation on the Gene Expression of Liver Lipid Metabolism in Dairy Goat Bucks

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Abstract: This study aimed to investigate how diets supplemented with DHA-rich algae affect the expression of liver lipid synthesis genes in dairy goat bucks. The results revealed that when supplemented with DHA-rich algae, liver weight and serum HDL-C were significantly increased ($p < 0.05$), as well as serum LDL-C was significantly decreased ($p < 0.05$). Transcriptome sequencing indicated that algae supplementation alters liver gene expression. The differentially expressed genes were predominantly enriched in fatty acid metabolism and the biosynthesis of unsaturated fatty acids. The expression of fatty acid desaturation and transcription factors (*SCD*, *FADS1*, *INSIG1*), de novo synthesis fatty acids (*FASN*), fatty acid transport (*LDLR*), and cholesterol and steroid synthesis (*HMGCR*, *HMGCS1*, *SQLE*) genes were significantly increased ($p < 0.05$), and fatty acid oxidation (*ALDH3B1*) genes were significantly decreased ($p < 0.05$). In conclusion, this research provided preliminary evidence that supplementation with algae in dietary supplements altered the expression of the liver lipid synthesis genes in the Saanen dairy goat bucks.

Keywords: algae; RNA-Seq; liver; lipid metabolism; dairy goat bucks

Citation: Ni, M.; Zhang, Z.; Luo, X.; Tian, M.; Zhu, Y.; Song, M.; Lei, H.; Chen, Z.; Li, C. Effect of Algae Supplementation on the Gene Expression of Liver Lipid Metabolism in Dairy Goat Bucks. *Agriculture* **2024**, *14*, 685. https://doi.org/10.3390/agriculture14050685

Academic Editors: František Zigo, Jana Výrostková, Mária Vargová and Secundino López

Received: 8 February 2024
Revised: 19 April 2024
Accepted: 25 April 2024
Published: 27 April 2024

1. Introduction

Recognized as a distinguished breed among dairy goats, Saanen dairy goats are characterized by high milk yield, high fertility, and disease resistance. Additionally, they possess significant value in the ability to enhance the meat quality of their kids. Dairy goat bucks are mostly used for fattening meat, with only a small number of them retained for breeding purposes. After birth, young dairy goat bucks undergo a period of rapid growth and development, requiring significant nutritional support. Conventional buck feeds are insufficient for optimal growth and development, necessitating the search for nutrient-rich, functional protein feed alternatives. Proper feed composition and optimal nutrient intake in daily feeding management can significantly accelerate the growth and development of goats.

Algae, rich in nutrients, contain bioactive polysaccharides beneficial for animal health and growth, along with essential trace elements and vitamins [1], and the use of algae in feed additives for ruminants is gaining increasing popularity. Carbohydrates constitute 70% of the dry matter (DM) of algae, while the lipid fraction is typically less than 5% [2]. Previous research has demonstrated that incorporating algae dietary supplements into animal feeds positively influences animal growth and health [3]. Specific nutrients derived from algae, such as sulfated polysaccharides, green tannins, diterpenes, and DHA, have been observed to increase meat quality and growth performance [4].

The nutritional value, biological activity, and rapid growth rate of algae render them potentially valuable as feed and feed ingredients. This supplementation can enhance animal health, which, in turn, improves the quality of the fresh meat [5]. Incorporating kelp proteins and fucoidan into pig diets has been demonstrated to increase growth performance [6]. Furthermore, prebiotic components from brown algae may aid in managing stress in piglets by enhancing gut health and immune function post-weaning [7]. Supplementing fucoidan to sub-par forage rations improved feed digestibility in Holstein cows [8]. Additionally, a significant increase in milk fatty acid concentration was observed in dairy cows fed algae dietary high in docosahexaenoic acid (DHA increased from 0.04 to 0.91 g/100 g, and C18:2 cis-9, trans-11 increased from 0.36 to 1.87 g/100 g) [9]. Variations were noted in protein digestibility and dietary nitrogen intake when different species of algae were introduced into sheep diets [10].

Extensive research has been undertaken to study the role of algae on growth performance, meat quality, and feed digestibility in cattle and pigs, while limited research has concentrated on the effect of algae on dairy goats. In this study, we added 3% algae to the diet of 6-month-old Xinong Saanen dairy goat bucks. We conducted feeding trials and measured various aspects of growth performance, as well as performing liver and serum biochemical index assays and liver transcriptomics. Therefore, this study aims to preliminary gain useful insights into the use of algae in dairy goats by studying how supplementing their diet with algae affects their growth performance and the expression of liver lipid synthesis genes in bucks.

2. Materials and Methods

2.1. Ethics Approval and Consent to Participate

All experimental procedures were performed under the approval of the Institutional Animals Care and Use Committee (IACUC) of the Northwest A&F University, China (Approval No. DK2021054).

2.2. Animals and Treatments

Xinong Saanen dairy goats were bred at the Northwest A&F University through the crossbreeding of the native Saanen dairy goat from western Bern, Switzerland, with the local Guanzhong goat breed in Shaanxi. A one-way randomized experimental design was employed to select 24 dairy goat bucks (21.9 $\pm$ 0.98 kg) of 6-month-old Xinong Saanen, which were randomly divided into a control group and an algae group, and four bucks were randomly selected from each group for subsequent RNA-seq. Four biological replicates were used in each group. Each biological replicate contained one goat. The goats in the control group were fed a basal ration, and the goats in the alage group were fed a basal ration with 3% algae (dry matter basis). Following a 5-day acclimatization period, the formal trial lasted for 12 weeks.

The diets were formulated based on the Nutritional Requirements of Dairy Goats (NRC 2007) to fulfill the nutritional needs of the experimental goats. The basal ration consisted of a total mixed ration (TMR) with a concentrate-to-crude ratio of 3:7. Algae (*Schizochytrium* sp.; EE $\geq$ 60%, DHA $\geq$ 12%; product All-G Rich based on algae *Schizochytrium* spp.; Alltech Inc., Nicholasville, KY, USA), which was precisely weighed and thoroughly mixed before each feeding [11]. The composition of the algae contents and basal ration can be found in Tables 1 and 2.

The test animals were housed in a single pen measuring 2.2 m long and 2.95 m wide, with an exercise yard measuring 4.95 m long and 3.35 m wide. The experimental period followed the routine operating procedures of the goat farm, and the barn was disinfected once a month using 0.5% chlorothalonil to maintain the health and disease-free status of all experiment bucks. The bucks were granted unrestricted access to feed and water, available at their leisure, with feeding occurring twice daily at 08:00 and 16:00 throughout the trial period.

Table 1. Main nutrient content in algae (%, dry matter basis).

Items	Content	Amino Acid	Content	Vitamin	Content
dry matter	95	aspartate	1.20	vitamin C	0.71
crude protein	8–10	glutamate	0.38	vitamin B_1	0.0074
ether extract	55–60	alanine	0.31	vitamin E	0.0007
DHA	18	glycine	0.22		
		threonine	0.81		
		isoleucine	0.25		
		leucine	0.30		
		phenylalanine	0.70		
		arginine	0.30		
		methionine	0.28		
		lysine	0.10		
		EAA	2.16		

Limiting amino acid: threonine, leucine, phenylalanine, arginine, and lysine. Essential amino acid (EAAs) contained threonine, isoleucine, leucine, phenylalanine, and lysine.

Table 2. Basal diet composition and nutrient levels (%, dry matter basis [1]).

Feeds	Contents	Nutrient Level	Contents
Corn grain	16.5	NE (MJ/kg) [3]	6.78
Soybean meal	7.5	OM	93.34
Wheat bran	3.6	CP	16.67
Rapeseed meal	0.9	EE	3.13
$CaHPO_4$	0.45	NDF	22.86
NaCl	0.45	ADF	7.54
Premix [2]	0.6		
Alfalfa hay	21		
Corn silage	49		
Total	100		

NE, net energy; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber. [1] DM was measured as 62.66%. [2] Premix is vitamin A 170,000 IU, vitamin D3 340,000 IU, vitamin E 300 IU, zinc 586 mg, iron 765 mg, manganese 350 mg, copper 256 mg per kg of diet. [3] The digestive energy is the calculated value, and the rest is the measured value.

2.3. Sample Collection and Biochemical Measurements

All bucks were euthanized by an overdose of isoflurane (Abbot, Chicago, IL, USA) and rapidly exsanguinated by severing the jugular vein and carotid artery at the time of the experiment's end. Organ weights were recorded, and the livers were immediately placed in liquid nitrogen for transport and subsequently stored at −80 °C in the laboratory. Whole blood were separated at 3000× *g* for 5 min at 4 °C. An ELISA Kit (Mlbio, Shanghai, China) was utilized to analyze the LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglyceride (TG), and total cholesterol (TC) in the serum.

2.4. RNA Extraction and Identification

The total RNA from the livers was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) following previous protocols [12]. RNA concentration and purity was measured using NanoDrop 2000 (Thermo, Waltham, MA, USA). RNA integrity was assessed by the Agilent Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA) [13].

2.5. Library Construction and Sequencing

For each liver sample, 1.0 µg of RNA was used. The RNA Library Preparation Kit (NEB, Ipswich, MA, USA) was employed to construct sequencing libraries following the previous protocol. Subsequently, sequencing of library preparations on the Illumina platform results in the generation of paired-end reads.

2.6. Transcript Sequencing and Processing

The raw data (short reads) obtained from transcriptional sequencing of liver tissue were processed using FASTP (version 0.21.1) with default parameters [14]. The index of ARS1.2 was generated through the HISAT2 (version 2.2.1) build software. Subsequently, clean data were aligned to ARS1.2 using HISAT2. Transcript abundance and gene abundance were evaluated based on the reads per million mapped reads (FPKM) and per kilobase fragment. The formula for this calculation is as follows:

$$\text{FPKM} = \frac{\text{cDNA Fragments}}{\text{Mapped Fragments(Millions)} \times \text{Transcript Length(kb)}}$$

2.7. Gene Function Annotation

The gene function annotation was conducted utilizing the following databases: clusters of homologous proteins (KOG), the KEGG Ortholog database (KO), and Gene Ontology (GO). The GO enrichment was conducted using the Wallenius non-central hypergeometric distribution [15]. The KEGG enrichment of DEGs was assessed using the KOBAS (version 3.0) software [16]. A corrected $p < 0.05$ was utilized as the threshold for the significant enrichment of GO entries and pathways.

2.8. Analysis of Differential Expression

The DEGs for two conditions were conducted using DEseq. DEseq employs statistical routines to determine the numerical gene expression data, utilizing models that are based on negative binomial distributions. The resulting *p*-values were adjusted using the Benjamini–Hochberg method. Genes identified by DEseq with an adjusted $p < 0.01$ were classified as differentially expressed. A threshold of FDR < 0.01 and $|\log_2(\text{fold change})| \geq 2$ was established for significant differential expression.

2.9. RNA Extraction and Quantitative Real Time PCR

The cDNA was prepared using a PrimeScript RT kit (Takara, Kusatsu, Japan). The RT-qPCR was performed on SYBR II (Takara, Kusatsu, Japan) to determine the mRNA expression of genes on a CFX96 (Bio-Rad, Hercules, CA, USA) following the previously published protocols [12]. Gene-specific primers (Table S1) were designed and synthesized by Sangon (Shanghai, China); these primers were optimized before the initial screening and quantitative analysis.

2.10. Statistical Analysis

All data were obtained from a minimum of three biological replicates. Data processing was performed using SPSS 26.0 software, and graphical representation was created using GraphPad Prism 8. The relative mRNA expression levels were normalized to *RPS9* and *UXT* with the $2^{-\Delta\Delta Cq}$ method. The results were presented as means ± SEM and assessed for statistical significance using ANOVA. *P*-values were considered significant as follows: * $p < 0.05$, ** $p < 0.01$.

3. Results

3.1. Effect of Dietary Algae Supplementation on Slaughter Performance and Serum Lipoproteins in Bucks

The weight of the buck livers in the algae group was significantly increased compared with the control group ($p < 0.05$), as shown in Table 3. The content of serum LDL-C and HLD-C was significantly increased in the bucks of the algae group ($p < 0.05$). Based on these results, we conducted RNA-seq in the livers to explore the impact of dietary algae supplementation on lipid metabolism in the bucks.

Table 3. Effects of DHA-enriched algae supplementation on slaughter performance and serum lipoprotein in bucks.

Items	Control Group	Algae Group	SEM	*p*-Value
Weight of heart (kg)	0.16	0.20	0.034	0.271
Weight of liver (kg)	0.54	0.70	0.057	0.049
Weight of spleen (kg)	0.04	0.06	0.009	0.192
Weight of kidney (kg)	0.10	0.11	0.012	0.356
Weight of lung (kg)	0.37	0.40	0.035	0.470
Weight of perirenal fat (kg)	0.70	0.68	0.035	0.535
Serum LDL-C (mmol/L)	17.04	14.97	0.915	0.034
Serum HDL-C (mmol/L)	37.00	45.22	3.382	0.023

3.2. Transcriptome Analysis of Algae Affecting Livers in Bucks

The quality control of the sequencing data of the liver tissues obtained from eight dairy goat bucks is shown in Table 4. A total of 58.62 Gb of clean data and 196.50 million clean reads with a Q30 percentage (those with a base quality > 30) over 93.51%, averaging 7.01 Gb of clean data per sample, were obtained. The proportion of GC content was in a reasonable range (48.94–50.32%). Approximately 94.31% of the reads were mapped to the dairy goat genome, and 84.85% were aligned to unique genome locations, as shown in Table 5.

Table 4. Quality control of sequencing data for eight bucks.

Samples	Clean Reads	Clean Bases	GC Content (%)	Q30 (%)
T1	23,494,800	7,009,186,736	49.76	93.86
T2	23,701,620	7,079,057,132	49.58	93.96
T3	24,113,819	7,189,863,470	50.03	94.02
T4	24,364,173	7,261,983,814	50.04	94.01
T5	25,269,054	7,536,447,072	50.32	94.18
T6	25,348,031	7,544,927262	48.94	93.96
T7	25,135,104	7,502,770,420	49.23	93.55
T8	25,078,200	7,492,937,914	49.33	93.51

Control group: T1, T2, T3, and T4; Algae group: T5, T6, T7, and T8.

Table 5. Sequence comparison between RNA-seq and reference genome of bucks.

Samples	Total Reads	Mapped Reads	Uniq Mapped Reads	Multiple Map Reads	Reads Map to '+'	Reads Map to '−'
T1	46,989,600	43,923,468 (93.47%)	39,596,781 (84.27%)	4,326,687 (9.21%)	21,355,131 (45.45%)	21,650,664 (46.08%)
T2	47,403,240	44,910,600 (94.74%)	40,550,406 (85.54%)	4,360,194 (9.20%)	22,127,403 (46.68%)	22,251,734 (46.94%)
T3	48,227,638	45,202,800 (93.73%)	40,495,665 (83.97%)	4,707,135 (9.76%)	21,975,966 (45.57%)	22,324,476 (46.29%)
T4	48,728,346	46,114,101 (94.64%)	41,702,845 (85.58%)	4,411,256 (9.05%)	22,735,756 (46.66%)	22,857,572 (46.91%)
T5	50,538,108	47,843,708 (94.67%)	42,941,732 (84.97%)	4,901,976 (9.70%)	23,554,459 (46.61%)	23,663,525 (46.82%)
T6	50,696,062	47,714,306 (94.12%)	42,671,441 (84.17%)	5042865 (9.95%)	23,400,402 (46.16%)	23,584,567 (46.52%)
T7	50,270,208	47,368,827 (94.23%)	42,634,390 (84.81%)	4,734,437 (9.42%)	23,368,077 (46.48%)	23,470,142 (46.69%)
T8	50,156,400	47,604,079 (94.91%)	42,881,641 (85.50%)	4722438 (9.42%)	23,434,383 (46.72%)	23,562,476 (46.98%)

Based on the normalized data, the expression of 25,409 genes was detected (including 9734 novel genes). The fragments per kilobase per million fragments (FPKM) are shown in Figure 1A. We investigated the Pearson's correlation of samples, as depicted in Figure 1B. We used principal component analysis (PCA) to cluster these two groups (Figure 1C). The selection of DEGs was performed with the general chi-squared test. A total of 48 unigenes were identified as DEGs between the control and alage groups, of which 28 were upregulated and 20 were downregulated (Figure 1D). The heatmap between the two groups indicates that algae supplements could greatly influence the transcriptome of the liver in bucks (Figure 1E).

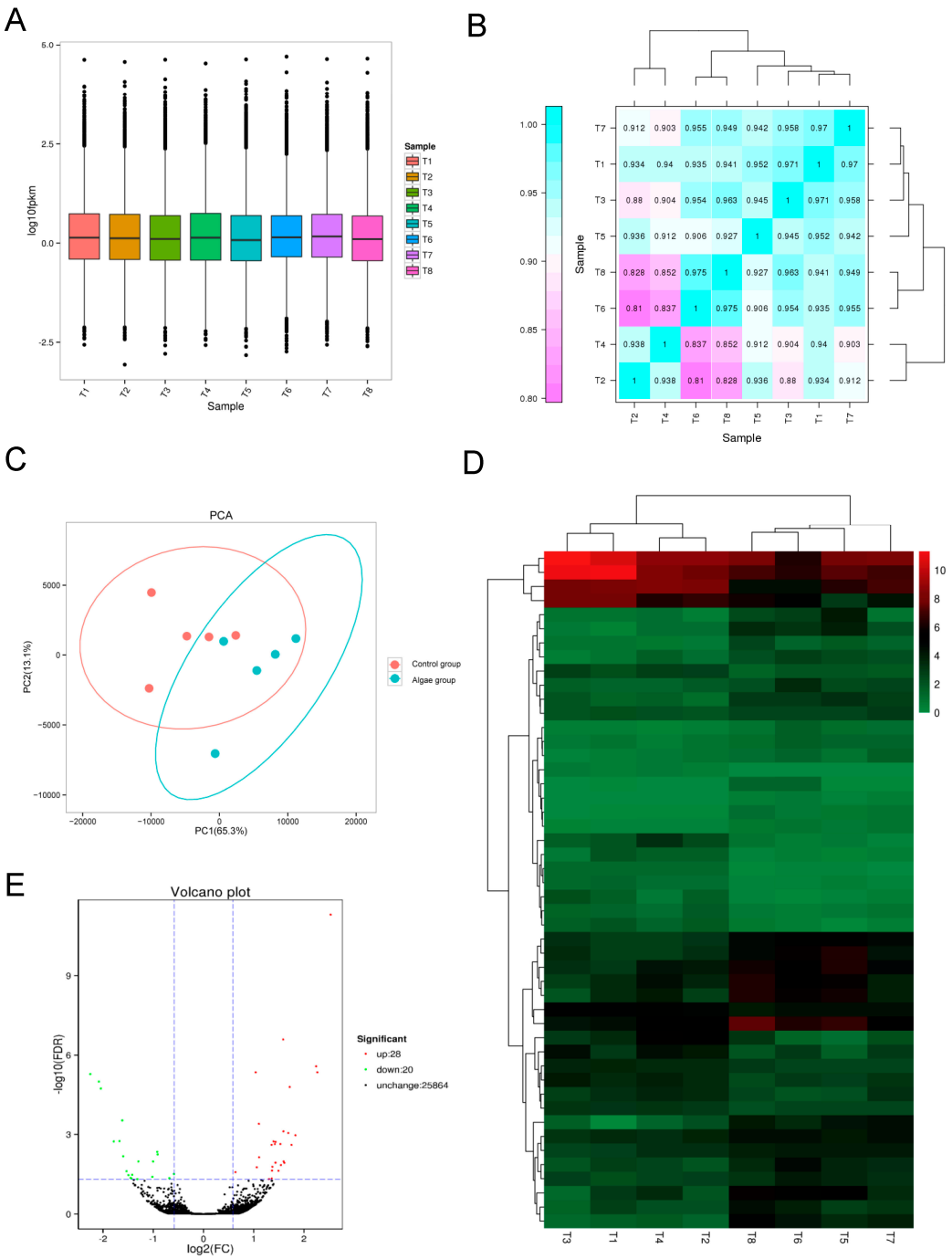

Figure 1. Algae affects the differential expression of liver genes in bucks. (**A**) FPKM. Expression quantity patterns of all genes in each sample. Moderately expressed genes accounted for the vast majority, and low- and high-expressed genes accounted for a small fraction. (**B**) Expression correlation

heat map of samples. (**C**) PCA plot from the control and algae groups on the Bray-Curtis distance. n = 4. In the graph, each dot signifies a specific sample, with the variation in dot colors denoting distinct groups. (**D**) The heat map of the relative expressions of DEGs. (**E**) Volcano plot from control and algae groups.

3.3. GO Term and KOG Enrichment Analysis

The GO and KOG classification enrichment analyses were conducted based on 48 DEGs. The GO annotation classification of DEGs is shown in Figure 2. These DEGs were enriched in the cellular process, biological regulation, single-organism process, metabolic process, multicellular organismal process, cellular component organization or biogenesis, localization, developmental process, multi-organism process, biological adhesion, and cell killing within biological process (BP); organelle, membrane, macromolecular complex, extracellular region, cell junction, and supramolecular complex in cellular component (CC); and the nucleic acid binding transcription factor activity, catalytic activity, binding activity, signal transducer activity, molecular transducer activity, and transporter activity in molecular function (MF).

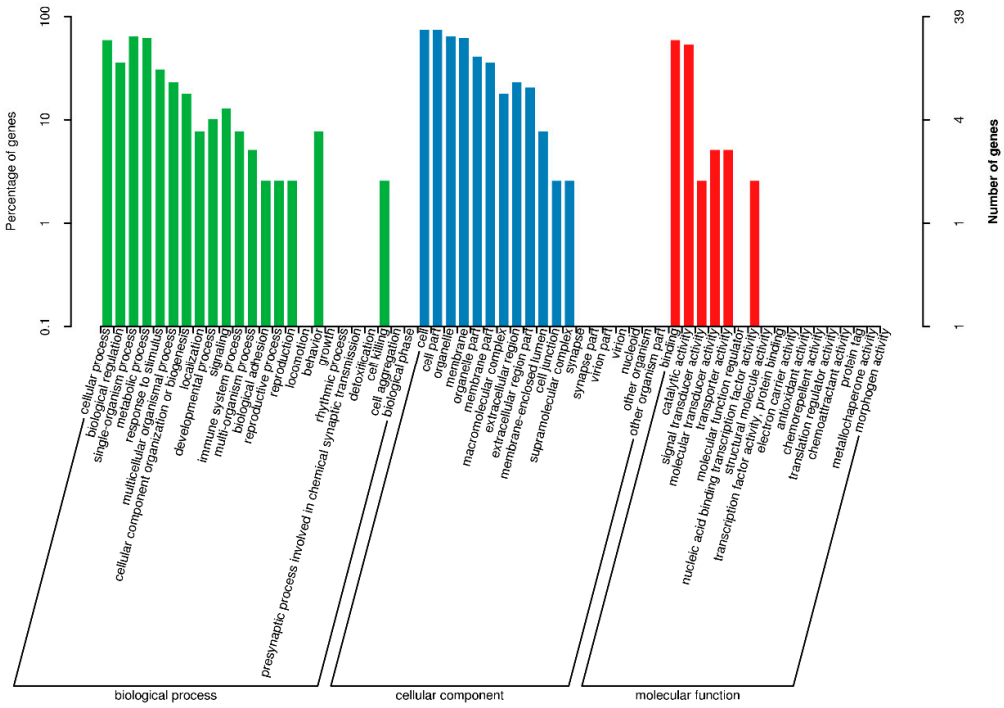

Figure 2. GO annotation classification of DEGs.

The KOG database annotated the DEGs for categorization and statistical analysis, with the results presented in Figure 3. The 48 DEGs were mainly enriched in lipid transport and metabolism (21.21%), general function prediction only (18.18%), and signal transduction mechanisms (15.15%).

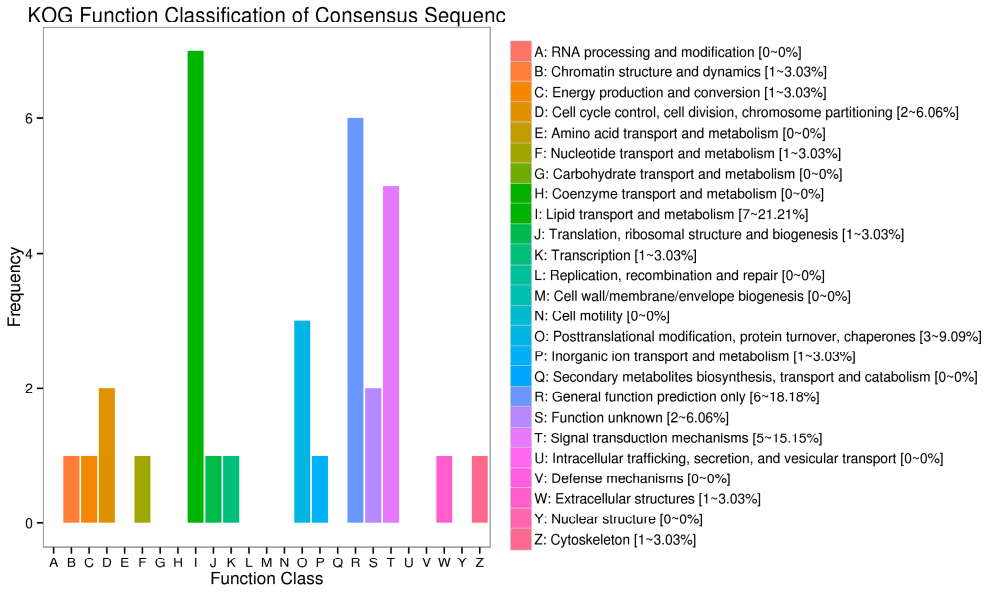

Figure 3. Statistical chart of KOG annotation classification of DEGs.

3.4. KEGG Pathway Analysis

As suggested by the KEGG pathway results (Figure 4), DEGs in the algae group were primarily enriched in fatty acid metabolism, biosynthesis of unsaturated fatty acids, B cell receptor, NF-κB, and primary immunodeficiency.

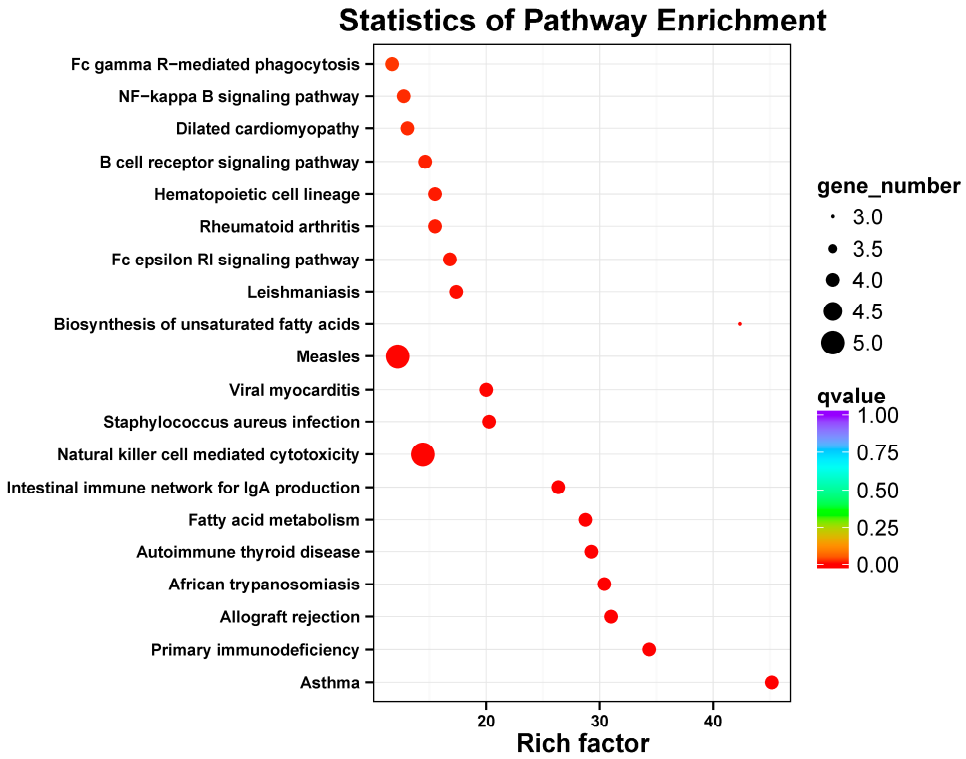

Figure 4. Scatter plot of KEGG pathways enriched by DEGs.

3.5. Screening of Key Genes for Dietary Algae Supplementation Affecting Lipid Synthesis in the Liver of Bucks

The GO, KOG, and KEGG enrichment revealed that algae influenced the gene expression related to liver lipid synthesis of bucks (Table 6). Importantly, fatty acid desaturation and transcription factors (*SCD*, *FADS1*, *INSIG1*), de novo fatty acid synthesis (*FASN*),

fatty acid activation and transport (*LDLR*), and cholesterol and steroid synthesis (*HMGCR*, *HMGCS1*, *SQLE*) genes were significantly increased ($p < 0.05$), and fatty acid oxidation (*ALDH3B1*) was significantly decreased in the algae group ($p < 0.05$). RT-qPCR was performed to verify the accuracy of the RNA-seq (Figure 5). Although the fold changes in the expression patterns of RNA-seq and RT-qPCR were slightly biased, this was probably owing to methodological differences. These findings confirm the reliability of our transcriptome results.

Table 6. Top ten candidate genes affecting lipid synthesis and metabolism in liver of bucks.

Gene	Count	Log_2 Fold Change	Regulated	FDR
SCD	2556.8	2.53	up	4.97×10^{-12}
LDLR	399.25	1.1	up	3.98×10^{-4}
FASN	609.25	1.59	up	7.67×10^{-4}
FADS1	1441.9	1.42	up	2.31×10^{-3}
INSIG1	3975.9	1.53	up	2.31×10^{-3}
FADS2	1227.6	1.6	up	1.05×10^{-2}
HMGCR	1564.3	1.36	up	2.32×10^{-2}
SQLE	3953.9	1.36	up	4.60×10^{-2}
HMGCS1	8331.8	1.3	up	4.86×10^{-2}
ALDH3B1	62.25	−1.62	down	2.96×10^{-4}

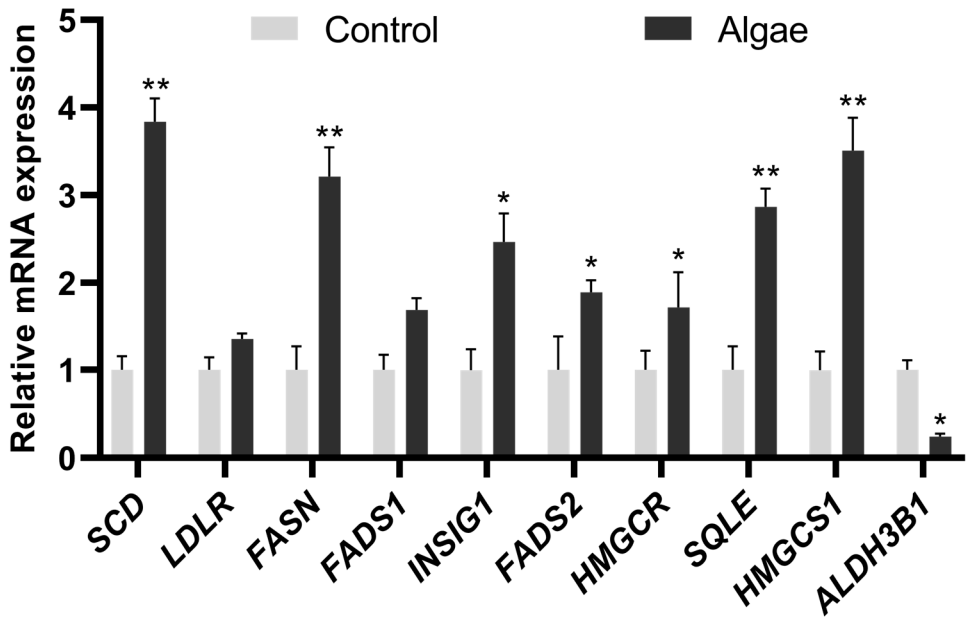

Figure 5. Real-time quantitative PCR verification results for top ten candidate genes. *p*-values were considered significant as follows: * $p < 0.05$, ** $p < 0.01$.

4. Discussion

Several studies have shown that the supplementation of feed with algae improves carcass quality and increases muscle DHA concentrations [17,18]. In this study, the supplementation of algae to the feed significantly increased the weight of the livers in the bucks in the experiment. According to previous studies, incorporating 2% DM algae into the diet significantly improves fatty acid metabolism, while 3% DM DHA-algae enhances the meat quality in sheep [19,20]. In this study, the supplementation of algae (3% DM) to the feed increased DHA and n-3 PUFA levels significantly in muscle, as well as significantly increasing the liver weight. However, Urrutia et al. reported that adding 3.89% DM algae might reduce dietary palatability, consequently having the effect of decreasing feed intake and daily weight gain [21]. On the basis of the above results, it is hypothesized that

the supplementation of algae (3% DM) to the feed could significantly improve fatty acid metabolism in goat bucks, whereas algal additions above 3% DM may decrease feed intake and daily weight gain.

In this study, KEGG analysis revealed that 16 out of 22 significantly enriched pathways were related to disease and inflammation, indicating potential avenues for exploring the link between algae addition and disease. Notably, the KEGG analysis identified two significant pathways, both crucial for lipid transport, synthesis, and metabolism. Combining the results from GO, KOG, and KEGG analyses, we identified 10 DEGs that are significantly associated with liver lipid transport, synthesis, and metabolism. Among these genes, nine were found to be significantly upregulated, while the expression of the expression of the *ALDH3B1* gene was decreased. In Marchitti's study, it was observed that the *ALDH3* family plays a crucial role in defense against aldehydes and related oxidative processes [22]. However, the function and significance of *ALDH3B1* are not adequately understood, although it may be involved in defenses against oxidative stress, particularly lipid peroxidation-derived aldehydes. Hence, it is imperative to conduct further research to pinpoint the exact mechanism that leads to the downregulation of *ALDH3B1* expression.

Studies have shown a positive correlation between liver *FADS1* and *FADS2* expression and the DHA concentration in dairy goat muscle tissues [17]. The *FADS1* and *FADS2* genes are involved in synthesizing n-6 and n-3 PUFAs. Our study corroborated these findings, demonstrating that supplementation with algae significantly increased DHA levels in muscle and adipose tissues, and markedly upregulated liver *FADS1* and *FADS2* expression. However, lambs on diets supplemented with 3.89% DM algae exhibited no significant change in *FADS1* and *FADS2* expression in the subcutaneous adipose tissue, while it was downregulated in intramuscular adipose tissue [21]. There are possible explanations that include the variable expression of DHA-regulated genes in various tissues, the influence of dietary ALA from flaxseed, and findings that the expression levels of *FADS1* and *FADS2* by dietary DHA are influenced by the ratio of DHA to other fatty acids [17,23].

The *SCD* encodes stearoyl-CoA desaturase, primarily converting C18:0 to C18:1 c-9 [24–26]. It has been reported that polymorphisms in the *SCD* gene affect milk fat composition, notably impacting levels of palmitoleic acid, long-chain fatty acids, short-chain fatty acids, and the ratios of palmitoleic acid to palmitic acid [27]. *SCD* expression is regulated through dietary factors, particularly via n-6 and n-3 PUFA content, as well as via environmental and hormonal factors [28]. There was a negative correlation between the expression of *SCD* and the content of n-3 PUFA and DHA in beef cattle, indicating that algae-supplemented diets led to reduced *SCD* expression [29]. However, our results showed a significant upregulation in *SCD* expression, indicating a need for further investigation into its mechanisms.

FASN encodes fatty acid synthase, a critical protein regulating the synthesis of fatty acids [30]. *FASN* serves as a crucial enzyme in liver lipid synthesis and is linked to insulin resistance [31,32]. In cattle, *FASN* is located on *BTA19*, a region including several QTLs that exert a significant influence on the fat content of beef [33]. Our study found that adding 3% DM algae to the diet significantly increased *FASN* expression in dairy goat buck livers, paralleling Fougère's findings [34].

The research has indicated that adding algae to the diet disrupts cholesterol metabolism in rats and elevates *LDLR* gene expression in the liver, leading to reduced blood cholesterol levels [35]. This aligns with our observations that *LDLR* expression in goat liver was significantly enhanced following algae supplementation.

The overexpression of *INSIG1*, a regulator of lipid metabolism, significantly increased palmitic acid (C16:0) content in the mammary tissues of dairy goats, corroborating our prior findings [36]. Presumably, increased *INSIG1* expression in the liver would enhance the role of its encoded protein in fatty acid synthesis, leading to higher palmitic acid content in the organism's tissues.

As the agricultural production of dairy goats has become increasingly intensified, dairy goats have been exposed to a variety of external stressors, disturbing the redox balance

within the liver and resulting in oxidative stress. Algae is metabolized in the liver and has anti-inflammatory properties that were increased in mice, but the difference was not significant [37]. We hypothesize that the liver weight increase is due to the increased number of hepatic parenchymal cells and the activation of lipid synthesis. Therefore, subsequent studies should continue to focus on oxidative stress and its histomorphology in the liver. Inflammation is considered to be the most important innate immune system, which can defend against harmful stimuli. Algae contains primary and secondary bioactive molecules such as phenolic compounds, bioactive peptides, glycoproteins, polysaccharides, alkaloids, terpenes, and sterols, which have been proven to be an alternative to anti-inflammatory drugs [38,39]. This study demonstrates that incorporating algae into the diet of dairy goat bucks also has the effect of improving the immune response of the liver. As suggested by the KEGG pathway results, DEGs were enriched in the B cell receptor, NF-κB, and primary immunodeficiency in the algae group.

In summary, in the rumen, algae may be decomposed to short-chain fatty acids, with acetate and propanoate being transported to the liver through the serum, leading to alterations in serum LDL and HDL levels. The SCFA synthesis is catalyzed by *INSIG1* and *FASN*. Furthermore, SFA is desaturated into C22:6n3 catalyzed by *SCD*, *FADS1*, and *FADS2*. Ultimately, this affects the activation of genes involved in liver fat synthesis, indirectly resulting in a significant increase in liver weight. Based on all of the above discussion, a brief schematic model was produced that summarizes the impact of algae supplementation on lipid metabolism gene expression in the livers of dairy goat bucks (see Figure 6).

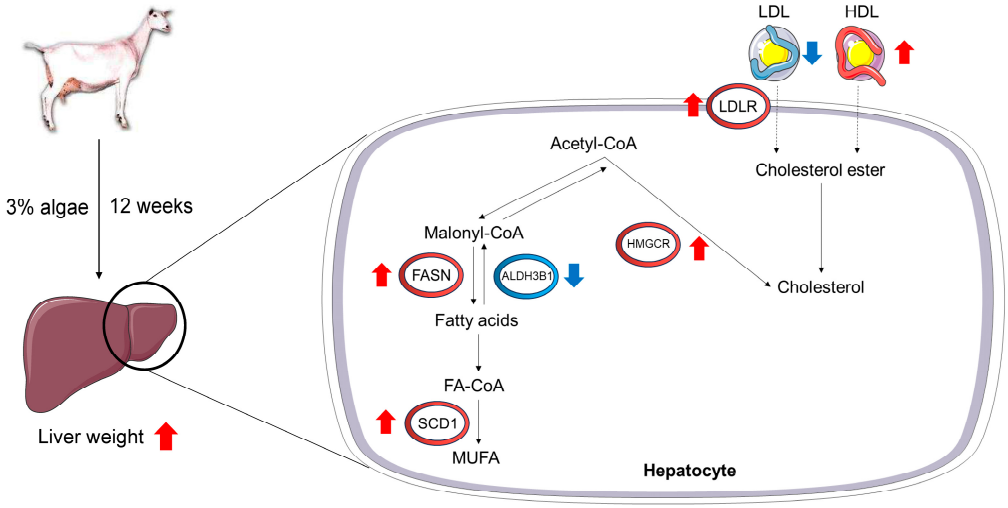

Figure 6. A model summarizing the effect of algae supplementation on the gene expression of liver lipid metabolism in dairy goat bucks (partly created by Servier Medical Art https://smart.servier.com/, accessed on 7 February 2024).

5. Conclusions

In this study, supplementation with 3% algae in the diet significantly increased liver weight and serum HDL-C and significantly decreased serum LDL-C. Transcriptome sequencing of the liver exhibited an increase in liver lipid synthesis gene expression (*SCD*, *LDLR*, *FASN*, *FADS1*, *FADS2*, *HMGCR*, *SQLE*, and *HMGCS1*) and a decrease in those associated with lipid metabolism (*ALDH3B1*). In addition, in the algae group, GO, KOG, and KEGG analysis revealed that DEGs in the liver were mainly enriched in fatty acid metabolism, biosynthesis of unsaturated fatty acids, the B cell receptor, NF-κB, and primary immunodeficiency. Further research should focus on the key components in algae and liver lipid synthesis. This preliminary study showed that dietary supplementation with algae altered the gene expression of liver lipid metabolism in dairy goat bucks. Furthermore, a larger number of dairy goat bucks should be included in future studies to validate the findings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14050685/s1, Table S1. Primer pairs for RT-qPCR analysis.

Author Contributions: Writing original draft preparation, M.N., Z.Z., Y.Z. and M.S.; Data curation: X.L.; Validation: M.N.; Conceptualization: M.T.; Software: H.L. and Y.Z.; Supervision: Z.C.; Funding acquisition: C.L.; Methodology: M.S.; Writing—Reviewing and Editing: C.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was jointly supported by the "National Key Research and Development Program of China (2022YFD1300203)," the "Shaanxi Livestock and Poultry Breeding Double-chain Fusion Key Project (2022GD-TSLD-46-0203)," and the "Key Research and Development Program of Shaanxi Province (2018ZDCXL-NY-01-05)". The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Institutional Review Board Statement: All the experimental procedures were performed under the approval of the Institutional Animals Care and Use Committee (IACUC) of Northwest A&F University, China (Approval No. DK2021054).

Data Availability Statement: The datasets generated and/or analysed during the current study are available in the [NCBI gene expression omnibus database] repository. Accession Number: PRJNA1091031.

Acknowledgments: Figure 6 was partly generated using Servier Medical Art.

Conflicts of Interest: Author Huan Lei was employed by the company Xi'an Baiyue Goat Milk Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

DM, dry matter; DEGs, differentially expressed genes; TMR, total mixed ration; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; TG, triglycerides; TC, total cholesterol; FPKM, Fragments Per Kilo bases per Million fragments; PCA, principal component analysis; RT-qPCR, quantitative real time PCR; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid, MUFA, monounsaturated fatty acid; *SCD*, stearoyl-CoA desaturase; *LDLR*, low density lipoprotein receptor; *FASN*, fatty acid synthase; *FADS1*, fatty acid desaturase 1; *FADS2*, fatty acid desaturase 2; *INSIG1*, insulin induced gene 1; *HMGCR*, 3-hydroxy-3-methylglutaryl-CoA reductase; *SQLE*, squalene epoxidase; *HMGCS1*, 3-hydroxy-3-methylglutaryl-CoA synthase 1; *ALDH3B1*, aldehyde dehydrogenase 3 family member B1; *RPS9*, ribosomal protein S9; *UXT*, ubiquitously expressed prefoldin like chaperone.

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