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Dietary curcumin supplementation regulates the lipid metabolism in laying hens

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ABSTRACT

This study was conducted to investigate the effects of dietary curcumin on the hepatic lipid metabolism and antioxidant capacity in Roman laying hens. A total of 144 Roman laying hens (28 weeks old) were allocated 4 dietary treatments that included curcumin at concentrations of 0, 50, 150, and 250 mg/kg for 9 weeks. Each treatment had 6 replicates per group with 6 hens per replicate (2 birds/cage). The results showed that dietary supplementation of curcumin decreased ($P < 0.05$) the absolute and relative abdominal fat weight during 32–34 wk. Curcumin supplemented at 150 mg/kg had been reduced ($P < 0.05$) the concentrations of triglycerides (TG) and total cholesterol in plasma, the liver TG concentrations, and the enzyme activities of malic (ME) and lipoprotein lipase of Roman laying hens throughout the experiment. Curcumin supplemented also decreased ($P < 0.05$) the mRNA expression of Sterol expression-regulatory protein-1c (SREBP-1c), *ACC*, *FAS*, *ME* and while it increased the mRNA expression of Apolipoprotein B-100 and Apolipoprotein-II. Further research found that dietary curcumin boosted the activity of the antioxidant enzymes Superoxide dismutase, Catalase, and Glutathione peroxidase in plasma and liver. It lowered the levels of Malondialdehyde in plasma and liver. In conclusion, curcumin inclusion regulated the hepatic fat metabolism, reduced abdominal fat deposition and improved the antioxidant capacity of Roman laying hens.

HIGHLIGHTS

- Curcumin can regulate the lipid metabolism of Roman laying hens by reducing the activity of lipid metabolism enzymes in the liver.
- Curcumin can regulate the fat deposition of Roman laying hens by reducing the expression of genes involved in lipid synthesis in the liver.

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Curcumin; roman laying hens; liver; lipid metabolism

Introduction

In recent years, with the rapid development of modern farming, the laying hens are commonly grown in cages (Mench et al. 2011; Mench and Rodenburg 2018). As a result of long-term confinement in cages, the laying hens have lacked the free movement ability. It leads to reduced energy consumption, making it more likely to have obesity, fatty liver, and other metabolic diseases (Shini et al. 2019), which culminate in decreased production and less economic farming. Therefore, understanding the process of lipid metabolism of laying hens and the improvement of the physiological state of layers is essential in promoting egg production.

The liver is a vital organ for regulating lipid metabolism in chickens. The liver synthesises more than 90% of fatty acids, which are then incorporated into Triglycerides and transferred to other tissues in the form of Lipoproteins for usage or storage (Hermier 1997; Laliotis et al. 2010). The liver synthesises the majority of the fat deposited in adipose tissue (Mench and Rodenburg 2018). Furthermore, the absorption of nutrients by chickens differs from that of mammals. The fat absorbed by the gastrointestinal tract enters the hepatic portal system via the portal vein and is transported to the liver, where it is metabolised (Shini et al. 2019). Due to the high need for lipids in the egg production process (Emami et al. 2020), the liver of laying hens plays a vital role in controlling fat

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Table 1. Composition of basal diets (as-fed basis).

Ingredients	Content	Nutrient levels	Content
Corn	64.5	ME/(MJ/kg)[2]	11.42
Soybean meal	18	CP	16.42
Wheat bran	2	Ca	3.73
Fish meal	5	TP	0.41
Limestone	9	Met	0.31
$CaHPO_4$	1	Cys	0.27
NaCl	0.1	Lys	0.97
Premix[1)]	0.4		

Note: The premix provided, per kg of diet: Vitamin A 9000 IU; Vitamin D 2500 IU; Vitamin E 20 IU; Vitamin B 1212 μg; Vitamin K 2.4 mg; Mn 100 mg; Zn 60 mg; Fe 25 mg, Cu 5 mg; Co 0.1 mg (Mn, Zn, Fe, Cu, Co were provided in the form of sulfates); Se (Na2SeO3.5H2O) 0.2 mg; I (KI) 0.5 mg. Values were determined except ME.
ME: Metabolic energy; CP: Crude protein; TP: Total phosphorus.

metabolism. When the amount of fatty acid absorption and de novo synthesis exceeds the amount of fatty acid consumption, the excess Triglycerides accumulate in the liver and adipose tissue, resulting in fatty liver. Excess Triglycerides may also be deposited in the abdomen, which has a negative impact on the health and egg production of laying hens, as well as their egg production.

Curcumin is a natural plant spice extracted from turmeric rhizomes (*Curcuma longa L*). Previous studies suggested that curcumin is a functional molecule having antioxidant (Galli et al. 2018), anti-inflammatory (Johannah et al. 2018), antibacterial (Lee et al. 2013), and immunostimulant properties (Hossein and Hossein 2016). It is commonly used in animal feed to increase animal health and productivity. Curcumin has been shown to lower plasma cholesterol, triglycerides, and low-density lipoprotein levels in mice fed a high-cholesterol diet (Shin et al. 2011). Moreover, curcumin can inhibit chronic inflammation, reduce fat deposition, and reduce the negative health effects of obesity (Shao et al. 2012). Curcumin has been demonstrated to be an effective regulator of insulin Receptor Substrate-1 and Nuclear Ubiquitin I, inhibiting the Peroxisome Proliferator-activated Receptor (PPAR) pathways, limiting fatty acid production (Ye et al. 2014), and lowering the blood lipid contents of animals (Ejaz et al. 2009; Nouzarian et al. 2011). Previous research has shown that feeding curcumin to Roman laying hens can protect ovaries and increase egg-laying performance (GAO 2020). However, the effect of dietary curcumin supplementation on fat metabolism in Roman laying hens remains unknown. Therefore, in this study, the effects of dietary curcumin on blood lipids, hepatic fatty acid synthase activity and the expression of related genes in Roman laying hens were analysed, and the effects of dietary curcumin on hepatic lipid metabolism in Roman laying hens were revealed.

Materials and methods

Ethical approval and experimental chickens housing and management

The present study was performed on the experimental poultry house of Guangdong Ocean University (Zhanjiang, Guangdong, China), and the Animal Care and Use Committee of Guangdong Ocean University approved all experimental procedures. Curcumin was purchased from Xi'an Golden Green Biotechnology Co., Ltd. ($\geq$ 95%). A total of 144 twenty-eight-week-old Roman laying hens were obtained from Guangzhou Xiying Rare Poultry Breeding Co., Ltd. From 0 to 28 weeks of age the hens were fed on basal diets according to National Research Council (1994) and under the same environment in accordance with the Chinese chicken feeding standards (NY/T33-2004) (MAPRC, 2004). The 28 weeks old hens were randomly assigned to 4 treatments, each treatment with 6 replicates and each replicate with 6 chickens (2 birds/cage) for 9 wk of the experiment (from 28 to 37 weeks of age). The 4 dietary treatments included curcumin levels of 0, 50, 150, and 250 mg/kg. For the entire study, hens were kept in soft bedding cages in well-ventilated rooms. Cages and rooms have been sterilised with potassium permanganate and formalin. Artificial light was provided for 18 h/d until the end of the experimental period. Air conditioners and heaters were settled in the experimental chicken room to regulate the environmental temperature according to the body requirement of hens. The temperature was maintained at approximately 24 °C and the humidity was at 55–70%. Feed and water were offered on an ad-libitum basis. The hens were fed diets in mash form during the experimental 98 periods. Basal diets (Table 1) were based on the corn-soybean meal with composition and 99 nutrient levels consistent with the National Research Council (1994).

Sample collection

Sample collection was on Sundays at 31, 34, and 37 weeks of age of hens. Before sampling, all the hens fasted for 12 h and were weighed; six hens were randomly selected from each group ($n = 6$). The blood collection was performed from the brachial vein. The samples were stored in microtubes and then centrifuged at 3000 x g for 10 min at 4 °C to obtain the plasma, stored at −20 °C until analysis. Then, the

Table 2. The primer sequence and parameters.

Gene	Genebank	Primers	Primers sequences (5′→3′)	Product size
β-actin	NM_205518	Forward	CAACACAGTGCTGTCTGGTGGTAC	199bp
		Reverse	CTCCTGCTTGCTGATCCACATCTG	
ACC	NM_205505	Forward	AATGGCAGCTTTGGAGGTGT	136bp
		Reverse	TCTGTTTGGGTGGGAGGTG	
FAS	NM-205155	Forward	CGCAGTTTGTTGATGGTGAG	179bp
		Reverse	TCCTTGGTGTTCGTGACG	
ME	NM204303	Forward	TGCCAGCATTACGGTTTAGC	175bp
		Reverse	CCATTCCATAACAGCCAAGGTC	
SREBP-1c	AY029224	Forward	GCCCTCTGTGCCTTTGTCTTC	130bp
		Reverse	ACTCAGCCATGATGCTTCTTCC	
APOB-100	M18421	Forward	CACGCCTCACAGACCAAGTA	407bp
		Reverse	CCAGTCAAACGGCACATCTA	
APOVLDLII	NM-205483	Forward	AGGGCTGAACTGGTACCAACAAAC	140bp
		Reverse	GGATGACCAGCCAGTCACGA	

Note: the abbreviations are as follows: SREBP-1c: Sterol regulatory element-binding protein-1c; ACC: Acetyl CoA carboxylase; FAS: Fatty acid synthase; ME: Malate dehydrogenase; APOB-100: Apolipoprotein B-100; APOVLDL-II: Very Low-Density Apolipoprotein II.

chickens were slaughtered, and belly fat was taken out and weighed. The liver samples were weighted, chopped, separated, washed with ice-cold saline, and divided into 2 mL enzyme-free centrifuge tubes. Part of the liver tissue (g) and pre-chilled PBS (mL) or saline were fully homogenised at a ratio of 1:9 and then centrifuged at 8000 x g for 10 min at 4 °C to obtain the supernatant. The supernatant was stored in 2 mL plastic vials at −20 °C for further analysis. Part of the liver samples was transferred to the freezer (−80 °C) until gene expression analysis.

Plasma lipid analysis

Plasma Triglycerides (TG), Total cholesterol (TC), High-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C), and Very-low-density lipoprotein cholesterol (VLDL) contents were determined by the ELISA method and a Beijing Pulang DNM-9606 enzyme label analyser (Beijing, China). The ELISA kits were bought from the Jiangsu Meimian industrial Co., Ltd (Jiangsu, China) and the levels of TG, TC, HDL-C, LDL-C, and VLDL were measured according to the instruction manual.

Liver lipid and histological examination

Liver TG concentrations were measured using the final supernatant of plasma by ELISA method. For the histological examination, liver tissue was removed and immediately put soaked in 10% formaldehyde at room temperature for 24 h. Then, the liver tissue was dehydrated with conventional gradient alcohol and finally treated with xylene, and paraffin-embedded sections of 4 microns thickness were made. After HE was stained, the slices were observed for the histological changes in the liver under an ordinary optical microscope.

Liver enzymatic activities analysis

Liver enzymatic activity of Fatty acid synthase (FAS), Acetyl-CoA carboxylase (ACC), Malic enzyme (ME), and Lipoprotein lipase (LPL) were determined by the ELISA method and a Beijing Pulang DNM-9606 enzyme label analyser (Beijing, China). The ELISA kits were bought from the Jiangsu Meimian industrial Co., Ltd (Jiangsu, China) and measured according to the instruction manual.

RNA extraction and cDNA synthesis, and qPCR

Total RNA was extracted from the liver samples according to the total RNA extraction kits (Guangzhou Meiji Biological Technology Co., Ltd., Guangzhou, Guangdong). The integrity and quality of the extracted RNA were evaluated by 1% agarose gel electrophoresis and also were estimated using a 260/280 nm absorbance ratio (the range between 1.8 and 2.0 is an ideal ratio). The spectrophotometer (NanoDrop Lite, Gene Co., Ltd.) at 260 nm was used to study the total RNA level. Then, based on the protocol of the TransGen kit (Beijing TransGen Biotech Co., Ltd), the total RNA of each liver sample was used for cDNA synthesis by reverse transcription method. Then the qPCR was used to determine the mRNA expression level of Sterol expression-regulatory protein-1c (*SREBP-1c)*, *FAS*, *ACC*, *ME*, Apolipoprotein B-100 and Apolipoprotein-II. The primers (Table 2) were designed using Primer Premier 5.0 and synthesised by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). Quantitative analysis of specific gene mRNA expression was performed via qPCR by subjecting the cDNA obtained from the

Table 3. Effects of curcumin on liver development and abdominal fat deposition in laying hen.

Items	Time (week)	Dietary curcumin addition (mg/kg)				SEM	*P* Value	
		0	50	150	250		Linear	Quadratic
Average daily feed intake (g)	31	116.09	114.76	114.99	116.66	0.82	0.614	0.105
	34	113.16	114.52	116.85	117.71	2.68	0.220	0.928
	37	119.02	120.33	122.46	122.27	1.85	0.189	0.696
Absolute liver weight (g)	31	28.61	28.85	29.93	29.19	2.09	0.769	0.820
	34	31.66	31.07	30.50	32.18	1.80	0.904	0.546
	37	31.44	29.23	31.26	33.49	2.44	0.475	0.389
Relative liver weight (g)	31	2.05	2.30	2.27	2.24	0.19	0.530	0.474
	34	1.08	0.91	1.15	1.13	0.08	0.338	0.455
	37	1.38	1.09	1.10	1.34	0.09	0.784	0.020
Absolute abdominal fat (g)	31	21.67	22.24	17.19	19.49	2.51	0.333	0.739
	34	24.43^{a}	22.69^{a}	12.44^{b}	17.28^{ab}	2.93	0.042	0.294
	37	18.19	15.97	15.20	15.67	1.46	0.239	0.385
Relative abdominal fat (%)	31	1.55	1.75	1.31	1.49	0.18	0.470	0.985
	34	0.83^{a}	0.69^{ab}	0.44^{b}	0.54^{ab}	0.10	0.039	0.257
	37	0.66	0.55	0.54	0.55	0.07	0.286	0.410

Note: Data are expressed as the mean ($n = 6$), SEM means standard error, different lower-case letters on shoulder marks of the same row indicated significant differences ($P < 0.05$), while no letters or identical letters indicated no significant differences ($P > 0.05$).

above preparation to the Light Cycle instrument (Roche). The reaction mixture for qPCR (20 μL) contained 0.5 μL of each forward and reverse primer, 10 μL of Supermix, 2 μL of cDNA sample, and 7 μL of nuclease-free water. PCR products were analysed on 1% agarose gel to confirm the gene sequencing and specific temperatures. The reaction mixture (20 μL) for qRT-PCR contained 0.5 μL of each forward and reverse primer, 0.5 μL of Dye, 10 μL of Supermix, 1 μl of cDNA sample, and 7.5 μL of nuclease-free water. The qRT-PCR reactions were performed on a Bio-Rad CFX Connect real-time PCR detection system (Bio-Rad, Hercules, CA, USA). The qRT-PCR amplification conditions were as follows: pre-denaturation at 94 °C for 30 s, followed by 40 cycles of denaturation at 94 °C for 5 s, annealing at 55 °C for 15 s, and elongation at 72 °C for 10 s. The qPCR data were analysed from three replicates (each sample repeated three times); the transcript copy numbers were assessed in each group. The qPCR results were analysed using the 2-ΔΔCt method, and gene relative expression levels were expressed as a fold change to the internal control gene (*β-actin*).

Antioxidant index analysis

The activity of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH-Px) and Malondialdehyde (MDA) indicators in plasma and liver tissue homogenate were determined by the ELISA method and a Beijing Pulang DNM-9606 enzyme label analyser (Beijing, China). The ELISA kits were bought from the Jiangsu Meimian industrial Co., Ltd (Jiangsu, China) and measured according to the instruction manual.

Statistical analysis

Data were analysed using the GLM procedure of SAS 9.0 (SAS, 2009. SAS Institute Inc., Cary, NC) for a completely randomised block design. Replicates were the experimental units. Data were expressed as means. Differences among means were tested by using One-Way ANOVA and Tukey's test. (SPSS, 2004). Orthogonal polynomial contrasts were used to test the linear and quadratic effects of the increasing levels of dietary curcumin. Variability in data was expressed as the standard error of the mean, $P < 0.05$ was considered statistically significant, and $0.05 \leq P < 0.10$ was considered a tendency.

Results

Effects of curcumin on liver development and abdominal fat deposition in laying hens

The effects of curcumin on productive performance, liver development and abdominal fat deposition are shown in Table 3. During 32–34 wk, dietary supplementation of graded levels of curcumin showed a linear reduction in absolute and relative abdominal fat weight ($P < 0.05$). During 35–37 weeks, the curcumin addition groups' absolute and relative abdominal fat were reduced but without significant difference ($P > 0.05$). However, curcumin supplementation had no significant effect ($P > 0.05$) on average daily feed intake, absolute liver weight and relative liver weight throughout the experimental period.

Effect of curcumin on the concentrations of plasma lipids in laying hens

The effects of curcumin on the concentrations of plasma lipids are shown in Table 4. During 29–31 wk,

Table 4. Effect of curcumin on the concentrations of plasma lipids in laying hens.

Items	Time(week)	Dietary curcumin addition (mg/kg)				SEM	*P* Value	
		0	50	150	250		Linear	Quadratic
TG/(mmol/L)	31	7.25^{a}	6.85^{ab}	5.94^{b}	6.21^{ab}	0.35	0.032	0.368
	34	7.79^{a}	7.63^{ab}	6.54^{b}	7.20^{ab}	0.38	0.129	0.316
	37	8.34^{a}	7.56^{ab}	6.97^{b}	7.25^{ab}	0.34	0.035	0.161
TC/(mmol/L)	31	70.49^{a}	69.27^{ab}	58.03^{b}	65.99^{ab}	3.79	0.183	0.261
	34	73.63^{a}	72.28^{a}	59.96^{b}	64.71^{ab}	3.36	0.031	0.39
	37	79.88^{a}	75.08^{ab}	63.07^{b}	70.82^{ab}	4.31	0.076	0.183
LDL-C/(mmol/L)	31	7.36	7.23	6.74	7.14	0.38	0.521	0.503
	34	9.43	8.02	6.85	7.23	0.87	0.082	0.335
	37	8.42^{a}	7.22^{ab}	6.40^{b}	6.88^{ab}	0.58	0.07	0.187
HDL-C/(mmol/L)	31	1.28	1.26	1.39	1.4	0.08	0.194	0.813
	34	1.16	1.28	1.27	1.33	0.08	0.211	0.713
	37	1.28	1.26	1.27	1.37	0.09	0.542	0.528
VLDL/(mmol/L)	31	14.24	14.37	13.14	14.1	0.34	0.308	0.258
	34	13.67^{a}	13.35^{ab}	12.04^{b}	12.61^{ab}	0.43	0.049	0.333
	37	14.53^{a}	13.94^{ab}	11.56^{b}	12.96^{ab}	0.78	0.076	0.235

Note: TG, Triglyceride; TC, Total Cholesterol; LDL-C, Low density lipoprotein cholesterol; HDL-C, High density lipoprotein cholesterol, VLDL, Very low density lipoprotein. Data are expressed as the mean ($n = 6$), SEM means standard error, different lower-case letters on shoulder marks of the same row indicated significant differences ($P < 0.05$), while no letters or identical letters indicated no significant differences ($P > 0.05$).

dietary supplementation of graded levels of curcumin showed a linear reduction in plasma TG concentrations ($P < 0.05$). The concentrations of plasma TC were significantly ($P < 0.05$) decreased in the 150 mg/kg curcumin addition group. During 32–34 wk, the concentrations of plasma TG were significantly ($P < 0.05$) decreased in the 150 mg/kg curcumin addition group. The dietary supplementation of graded levels of curcumin showed a linear reduction in plasma TC and VLDL concentrations ($P < 0.05$). The dietary supplementation of graded levels of curcumin tended to linearly decrease the concentrations ($P < 0.10$) of plasma LDL-C but no significance ($P > 0.05$). During 35–37 wk, dietary supplementation of graded levels of curcumin showed a linear reduction in plasma TG concentrations ($P < 0.05$) and tended to linearly decrease ($P < 0.10$) the concentrations of plasma TG, LDL and VLDL.

Effects of curcumin on the liver TG concentrations and the activity of lipid metabolism enzymes in laying hens

The effects of curcumin on the liver TG concentrations and the activity of lipid metabolism enzymes are shown in Table 5. During 29–31 wk, dietary supplementation of graded levels of curcumin showed a linear reduction in liver TG concentrations and the enzyme's activity of ME and LPL ($P < 0.05$). Dietary supplementation of graded levels of curcumin showed a linear and quadratic reduction in the enzyme's activity of ACC ($P < 0.05$). During 32–34 wk, dietary supplementation of graded levels of curcumin showed a linear reduction in liver TG concentrations and the enzyme's activity of ME and LPL ($P < 0.05$). During 35–37 wk, dietary supplementation of graded levels of curcumin showed a linear reduction in liver TG concentrations and the enzyme's activity of LPL ($P < 0.05$). The enzyme activity of FAS and ME were significantly decreased ($P < 0.05$) in the 150 mg/kg curcumin addition group.

Effects of curcumin on liver histology in laying hens

The effects of curcumin on the liver histology are shown in Figures 1–3. Compared with the NC group, the liver tissue of the curcumin-treated group was tightly structured, the cell morphology and structure were normal, and the cells were well developed. The cells and sizes are the same, and there are no vacuoles between the tissues.

Effects of curcumin on the expression of lipid metabolism genes in laying hens

The effects of curcumin on the mRNA expression levels of different genes are shown in Table 6. During 29–31 wk, dietary supplementation of graded curcumin levels tended to linearly decrease the mRNA expression of the *SREBP-1c*. Dietary supplementation of graded levels of curcumin showed a linear and quadratic reduction in the mRNA expression of *ACC* and *FAS* ($P < 0.05$). Dietary supplementation of graded levels of curcumin showed a linear reduction in the mRNA expression of the *ME*, a linear increment in the mRNA expression of the *APOB-100* and a quadratic increment in the mRNA expression of the *APOVLDLII* ($P < 0.05$). Consumption of curcumin supplements for 32–34 weeks resulted in a linear reduction (P 0.05) in the mRNA expression of *SREBP-1c* and *ME* and linear and quadratic

Table 5. Effects of curcumin on the liver triglycerides (TG) concentrations and the Activity of Lipid Metabolism Enzymes in laying hens.

Items	Time (week)	Dietary curcumin addition (mg/kg)				SEM	*P* Value	
		0	50	150	250		Linear	Quadratic
Liver TG/(mmol/L)	31	1.46a	1.43ab	1.34b	1.37ab	0.03	0.04	0.404
	34	1.38a	1.34ab	1.22b	1.29ab	0.04	0.042	0.149
	37	1.37a	1.31ab	1.26b	1.23b	0.03	0.007	0.619
ACC/(U/L)	31	143.67^{a}	139.81^{a}	139.18^{a}	119.96^{b}	2.2	< 0.001	0.008
	34	131.85	127.36	132.02	134.97	3.05	0.333	0.256
	37	131.85	127.36	132.02	134.97	3.05	0.333	0.256
FAS/(U/mL)	31	1830.61	1792.7	1805.08	1697.53	102.1	0.422	0.742
	34	1804.31^{a}	1771.81	1605.46	1695.99	92.81	0.271	0.526
	37	2512.40^{a}	2385.27^{ab}	2263.17^{b}	2389.04^{ab}	63.87	0.123	0.083
ME/(mIU/L)	31	2704.07^{a}	2624.23^{ab}	2452.25^{b}	2490.64^{ab}	75.59	0.043	0.457
	34	2716.36^{a}	2608.87^{ab}	2476.82^{b}	2482.96^{ab}	73.09	0.034	0.459
	37	2733.50^{a}	2685.62^{ab}	2586.95^{b}	2659.50^{ab}	43.84	0.141	0.207
LPL/(U/L)	31	618.25^{a}	587.53^{a}	593.48^{a}	543.94^{b}	11.94	0.004	0.453
	34	604.38^{a}	580.60^{ab}	508.27^{b}	505.30^{bc}	22.35	0.006	0.654
	37	604.38^{a}	580.60^{ab}	508.27^{b}	505.30^{bc}	22.35	0.006	0.654

Note: ACC, Acetyl CoA carboxylase; FAS, Fatty acid synthetase; ME, Malic enzyme peroxidase; LPL, Lipoprotein lipase. Data are expressed as the mean ($n = 6$), SEM means standard error, different lower-case letters on shoulder marks of the same row indicated significant differences ($P < 0.05$), while no letters or identical letters indicated no significant differences ($P > 0.05$).

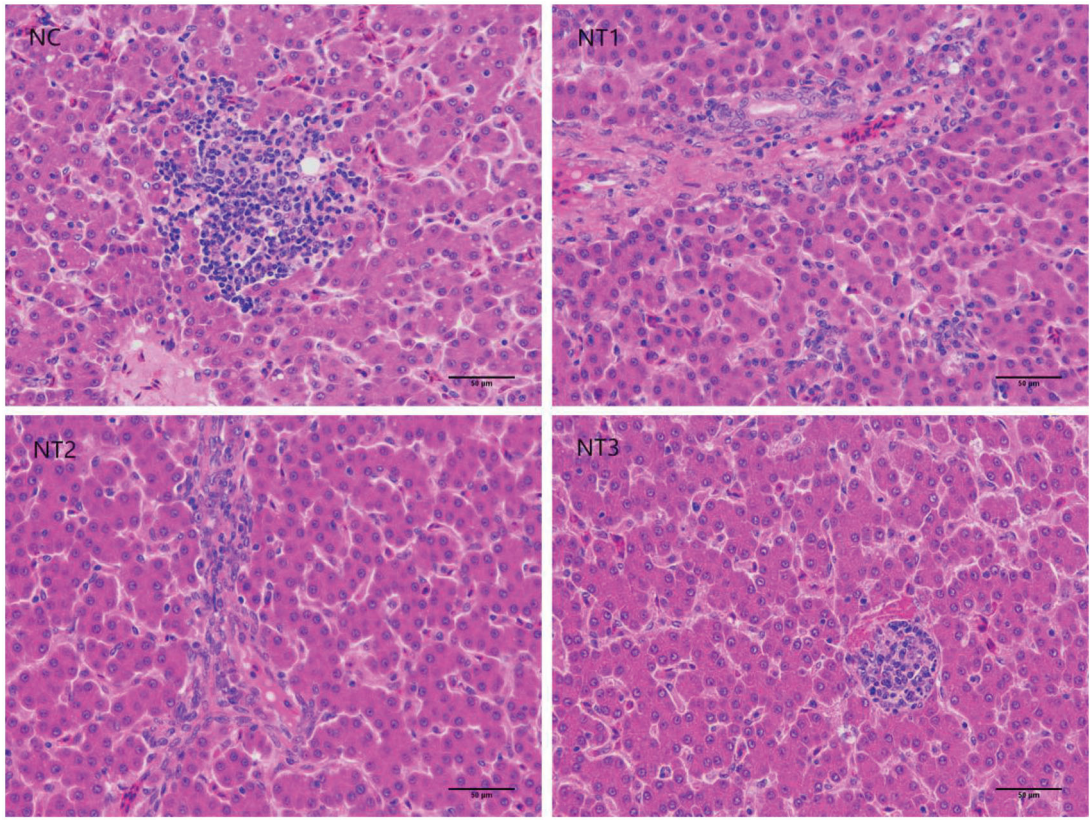

Figure 1. Effect of curcumin on the liver structure of laying hens (31 wk, H.E, 40×). Scale bar= 50 μm (H.E: Hematoxylin-eosin staining).

reductions in *ACC* and *FAS*, respectively. The mRNA expression of the *APOB-100* increased linearly (*P* 0.05), and the mRNA expression of the *APOVLDLII* increased quadratically (P 0.05) following dietary treatment with graded amounts of curcumin. During 35–37 wk, dietary supplementation of graded levels of curcumin showed a linear reduction ($P < 0.05$) in the expression of *SREBP-1c* and *ACC* genes. Dietary supplementation of graded levels of curcumin showed a linear and quadratic increment ($P < 0.05$) in the expression of the *APOB-100* gene and a linear increment in the expression of the *APOVLDLII* gene.

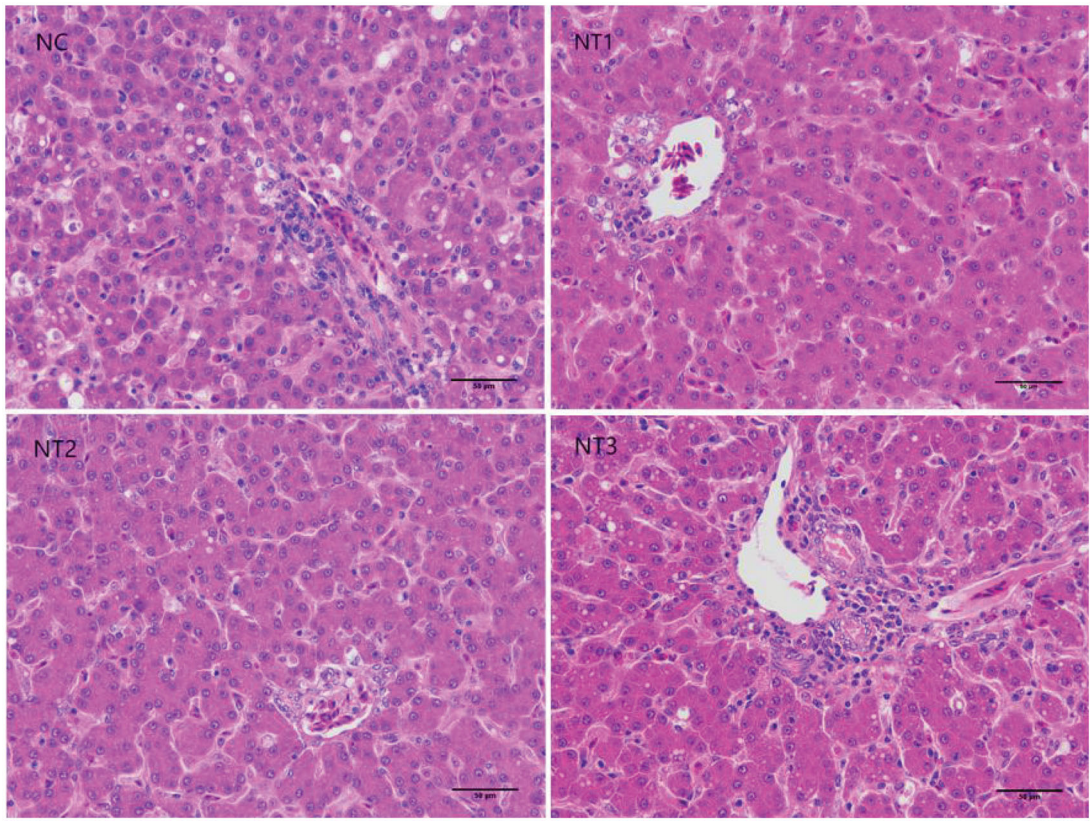

Figure 2. Effects of curcumin on the liver structure of laying hens (34 wk, H.E, 40×). Scale bar= 50 μm (H.E: Hematoxylin-eosin staining).

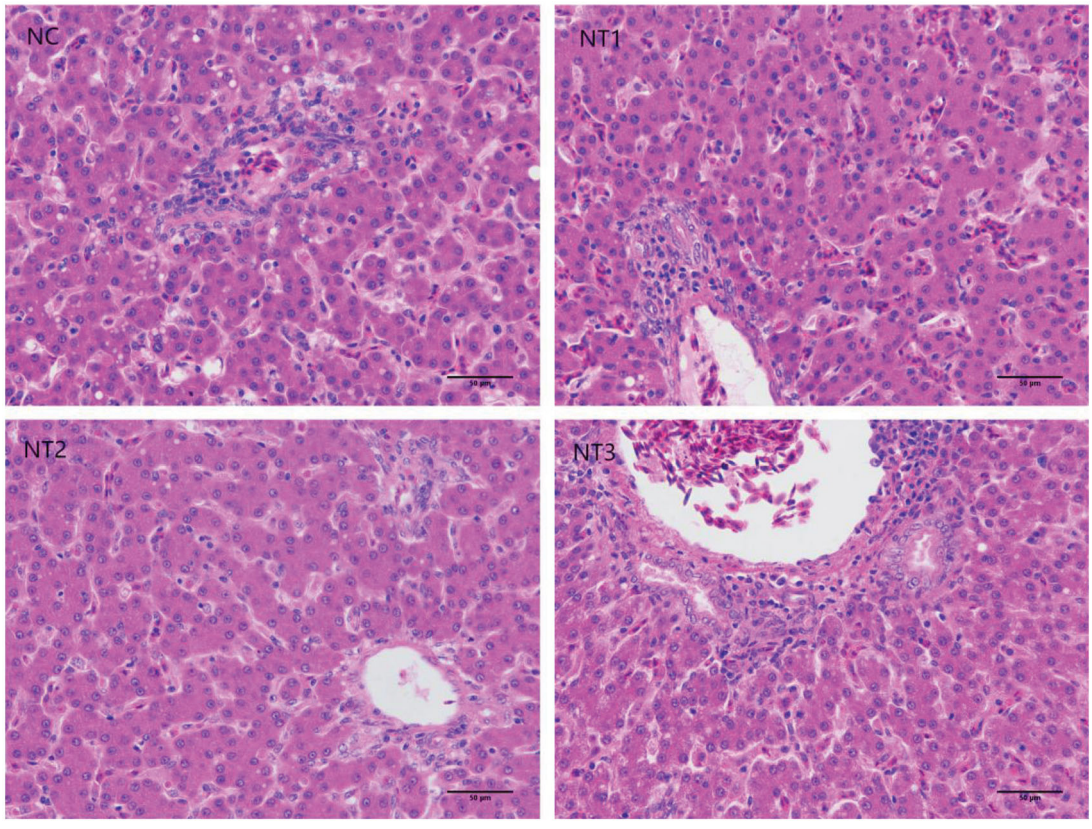

Figure 3. Effects of curcumin on the liver structure of laying hens (37 wk, H.E, 40×). Scale bar= 50 μm (H.E: Hematoxylin-eosin staining).

Table 6. Effect of curcumin on the expression of genes in the liver of laying hens.

Items	Time (week)	Dietary curcumin addition (mg/kg)				SEM	*P* Value	
		0	50	150	250		Linear	Quadratic
SREBP-1c	31	1.01^{a}	0.92^{a}	0.46^{b}	0.74^{ab}	0.14	0.079	0.214
	34	1.00^{a}	0.96^{a}	0.59^{b}	0.35^{c}	0.06	<0.001	0.14
	37	1.03^{a}	0.65^{ab}	0.38^{b}	0.38^{b}	0.15	0.012	0.249
ACC	31	1.02^{a}	0.67^{b}	0.44^{b}	0.69^{b}	0.10	0.023	0.014
	34	1.01^{a}	0.57^{b}	0.38^{b}	0.46^{b}	0.10	0.003	0.031
	37	1.00^{a}	0.85^{ab}	0.78^{ab}	0.58^{b}	0.10	0.015	0.826
FAS	31	1.01^{a}	0.93^{ac}	0.41^{b}	0.72^{c}	0.08	0.006	0.045
	34	1.00^{a}	0.59^{b}	0.49^{b}	0.73^{ab}	0.08	0.043	0.005
	37	1.03^{a}	0.67^{b}	0.85^{ab}	0.70^{ab}	0.11	0.122	0.370
ME	31	1.01^{a}	0.83^{ab}	0.61^{b}	0.69^{ab}	0.10	0.033	0.249
	34	1.00^{a}	0.88^{b}	0.77^{bc}	0.71^{c}	0.04	<0.001	0.366
	37	1.01	0.95	0.96	0.75	0.13	0.217	0.562
APOB-100	31	1.07^{b}	1.54^{ab}	1.57^{ab}	2.38^{a}	0.32	0.024	0.615
	34	1.03^{b}	1.60^{ab}	1.48^{ab}	2.02^{a}	0.21	0.016	0.944
	37	1.04^{c}	1.33^{bc}	3.56^{a}	2.07^{b}	0.30	0.005	0.019
APOVLDLII	31	1.06^{b}	1.61^{b}	2.90^{a}	1.50^{b}	0.26	0.056	0.006
	34	1.00^{b}	1.36^{b}	2.33^{a}	1.25^{b}	0.23	0.137	0.015
	37	1.03^{bc}	0.65^{c}	1.73^{ab}	2.38^{a}	0.23	<0.001	0.051

Note: SREBP-1c: Sterol regulatory element-binding protein-1c; ACC: Acetyl CoA carboxylase; FAS: Fatty acid synthase; ME: Malate dehydrogenase; APOB-100: Apolipoprotein B-100; APOVLDL-II: Very Low-Density Apolipoprotein II. Data are expressed as the mean ($n = 6$), SEM means standard error, different lower-case letters on shoulder marks of the same row indicated significant differences ($P < 0.05$), while no letters or identical letters indicated no significant differences ($P > 0.05$).

Table 7. Effect of curcumin on the plasma antioxidant enzyme activity of laying hens.

Items	Time (week)	Dietary curcumin addition (mg/kg)				SEM	*P* Value	
		0	50	150	250		Linear	Quadratic
SOD/(U/mL)	31	386.18^{b}	418.45^{b}	489.23^{a}	486.11^{a}	20.2	0.003	0.407
	34	405.96	417.41	464.25	448.64	20.08	0.087	0.519
	37	364.48^{bc}	391.08^{b}	451.28^{ab}	462.95^{a}	20.51	0.005	0.725
CAT/(U/mL)	31	8.12	8.75	9.31	9.06	0.43	0.113	0.336
	34	7.97^{b}	8.39^{ab}	9.62^{a}	9.36^{ab}	0.43	0.022	0.451
	37	7.76^{bc}	8.10^{b}	9.38^{a}	9.25^{ab}	0.39	0.011	0.565
GSH-Px/(U/L)	31	394.04	389.34	460.15	434.23	31.36	0.21	0.744
	34	364.55^{b}	400.05^{ab}	434.42^{a}	423.90^{a}	12.7	0.006	0.108
	37	397.34^{b}	420.99^{ab}	442.25^{ab}	459.51^{a}	17.97	0.032	0.863
MDA/(nmol/mL)	31	11.19^{a}	9.37^{ab}	8.18^{b}	9.23^{ab}	0.89	0.113	0.145
	34	12.64^{a}	11.45^{ab}	9.01^{bc}	9.42^{b}	0.83	0.01	0.79
	37	13.19^{a}	12.71^{a}	9.96^{b}	9.93^{b}	0.82	0.01	0.79

Note: SOD, Superoxide Dismutase; CAT, Catalase; GSH-Px, Glutathione peroxidase; MDA, Malondialdehyde. Data are expressed as the mean ($n = 6$), SEM means standard error, different lower-case letters on shoulder marks of the same row indicated significant differences ($P < 0.05$), while no letters or identical letters indicated no significant differences ($P > 0.05$).

Table 8. Effect of curcumin on the liver antioxidant enzyme activity of laying hens.

Items	Time (week)	Dietary curcumin addition (mg/kg)				SEM	*P* Value	
		0	50	150	250		Linear	Quadratic
SOD/(U/mL)	31	326.81	324.74	340.47	346.65	9.22	0.106	0.667
	34	325.52^{b}	317.27^{b}	340.21^{ab}	346.65^{a}	6.46	0.017	0.288
	37	362.99	368.33	392.64	381.15	11.08	0.15	0.469
CAT/(U/mL)	31	13.10^{b}	12.83^{b}	15.03^{a}	15.37^{a}	0.49	0.003	0.548
	34	21.87	21.13	22.07	22.49	0.49	0.231	0.262
	37	19.81	19.85	21.07	20.56	0.81	0.366	0.744
GSH-Px/(U/L)	31	153.27	161.91	182.44	191.81	12.96	0.047	0.978
	34	172.09^{b}	183.83^{ab}	198.74^{ab}	208.92^{a}	11.01	0.034	0.946
	37	168.51^{b}	184.56^{ab}	183.26^{ab}	198.57^{a}	8.43	0.046	0.966
MDA/(nmol/mL)	31	5.68^{a}	4.90^{ab}	5.16^{ab}	4.38^{b}	0.28	0.019	0.98
	34	9.55	9.33	8.82	8.93	0.35	0.172	0.651
	37	10.74^{a}	10.38^{ab}	9.15^{b}	8.96^{b}	0.39	0.006	0.826

Note: SOD, Superoxide Dismutase; CAT, Catalase; GSH-Px, Glutathione peroxidase; MDA, Malondialdehyde. Data are expressed as the mean ($n = 6$), SEM means standard error, different lower-case letters on shoulder marks of the same row indicated significant differences ($P < 0.05$), while no letters or identical letters indicated no significant differences ($P > 0.05$).

Effect of curcumin on the plasma and liver antioxidant index of a roman layer

The effects of curcumin on antioxidant capacity in plasma and liver are presented in Tables 7 and 8. During 29–31 wk, the activity of SOD in plasma was increased linearly as curcumin concentration increased, and the 150 mg/kg curcumin addition group was significantly decreased ($P < 0.05$) the plasma MDA contents. The activities of CAT and GSH-Px in the liver were increased linearly ($P < 0.05$) as curcumin concentration increased, and dietary supplementation of graded levels of curcumin showed a linear reduction ($P < 0.05$) in the MDA levels of the liver. During 32–34 wk, dietary curcumin addition tended to linearly increase ($P < 0.05$) the activity of SOD in plasma but no significance ($P > 0.05$). The activities of CAT and GSH-Px in plasma were increased linearly ($P < 0.05$) as curcumin concentration increased, and dietary supplementation of graded levels of curcumin showed a linear reduction ($P < 0.05$) in the MDA levels of plasma. The activities of SOD and GSH-Px in the liver were increased linearly ($P < 0.05$) as curcumin concentration increased. During 35–37 wk, the activities of SOD, CAT and GSH-Px in plasma were increased linearly ($P < 0.05$) as curcumin concentration increased, and dietary supplementation of graded levels of curcumin showed a linear reduction ($P < 0.05$) in the MDA levels of plasma. The activity of GSH-Px in the liver was increased linearly ($P < 0.05$) as curcumin concentration increased, and dietary supplementation of graded levels of curcumin showed a linear reduction ($P < 0.05$) in the MDA levels of the liver.

Discussion

Turmeric has long been considered an anti-inflammatory and health-promoting property, and Curcumin is one of turmeric's primary anti-inflammatory and health components (Emami et al. 2020). Previous studies have shown that dietary addition of curcumin can improve the antioxidant capacity of organs and tissues in broilers and laying hens and reduce the production of MDA concentrations (Rajput et al. 2014). This study also shows that feeding curcumin to Roman laying hens can linearly increase the activities of SOD, CAT and GSH-Px antioxidant enzymes in plasma and liver tissue and linear reduction the content of MDA in plasma and liver tissue. These results suggest that the addition of curcumin to the diet positively increases the antioxidant capacity of the body and tissues, which may improve the physiological status of laying hens by relieving the stress caused by production and activities during the caged laying process.

On the other hand, curcumin is currently thought to protect against the pathogenic effects of obesity and other metabolic illnesses and improve lipid metabolism (Aggarwal 2010). Pre-adipocytes and adipocytes can be inhibited by curcumin, which reduces the number of adipocytes and adipose tissue fat content. (Aggarwal 2010; Kim et al. 2011; Zhao et al. 2011). Curcumin can help livestock and poultry improve their physiological production status by regulating food intake and appetite, increasing the browning of white adipose tissue and inhibiting fat synthesis (Ejaz et al. 2009; Kang et al. 2011; Xie et al. 2019). Blood lipid levels are closely related to lipid synthesis and transport in the liver. Plasma TG and TC contents reflect lipid absorption, and HDL-C, LDL-C and VLDL reflect lipid decomposition and transport in vivo. Aggarwal B et al. showed that curcumin could regulate lipid metabolism and reduce lipid (Aggarwal 2010). Research by Nouzarian showed that turmeric powder could improve the feed utilisation efficiency of broilers and significantly reduce serum TG content (Nouzarian et al. 2011). This study showed that dietary supplementation of graded levels of curcumin could reduce the linear reduction of the concentrations of TG, TC and VLDL in plasma; among them, the addition amount of 150 mg/kg has a better effect.

Furthermore, for laying hens, fat synthesis mainly occurs in the liver. In the process of fatty acid synthesis, many essential enzymes, such as SREBP-1c, ACC, FAS, and ME, are required. FAS and ACC are critical components of cholesterol and triglyceride biosynthesis and are the rate-limiting enzymes for fatty acid production. ACC is responsible for catalysing the synthesis of malonyl-CoA. FAS is an enzyme that regulates the rate at which fatty acids are synthesised. NADPH provides all of the hydrogen required for fatty acid synthesis in the liver. In contrast, the NADPH required for fatty acid synthesis in the chicken liver is primarily provided through the pyruvate-malate pathway, including the NADP-malic enzyme (Tanaka et al. 1982). Research by Fan showed that adding 200 mg/kg of curcumin to the diet can significantly reduce the FAS and ACC enzyme activities in the liver of yellow-feathered broilers, thereby reducing liver lipid synthesis and achieving lipid-lowering effects (Fan et al. 2021). This study shows that the addition of 150 mg/kg and 250 mg/kg curcumin to the diet positively affects reducing FAS activities and ME in their livers and reduces liver TG contents of 29–37 wk Roman laying hens. It was shown that curcumin could affect the

synthesis rate of fatty acids by reducing the activity of fatty acid synthase in the liver of Roman laying hens, thereby reducing the synthesis of hepatic lipids and regulating lipid metabolism. LPL is also a key lipid metabolism enzyme in the liver, decomposing TG primarily to create fatty acids and glycerol (Goldberg 1996). As part of the traditional apoB-LP metabolism, LPL hydrolysis and other intravascular remodelling mechanisms rapidly transform VLDL into intermediate (IDL) and low-density lipoproteins, containing significant cholesteryl esters and lower fractional triacylglycerol content (Walzem et al. 1999). Bickerton A S T et al found that compared with plasma non-esterified fatty acids, fatty acids decomposed by LPL have an obvious absorption phenomenon, suggesting that LPL can promote the absorption of fatty acids, thereby promoting lipid storage in specific tissue cells (Bickerton et al. 2007). This study showed that the addition of curcumin could reduce the activity of LPL in the liver throughout the experiment, thus limiting the uptake of lipids by the liver.

This study also showed that curcumin addition positively reduces abdominal fat deposits in Roman laying hens. The accumulation of fat is also related to the change of mRNA expression of genes involved in fat synthesis and transport. According to reports, SREBP protein can directly stimulate the transcription of fatty acid-forming enzymes to regulate the biosynthesis of cholesterol and fatty acids in the liver (Brown and Goldstein 1997). *SREBP-1c* is one of the isomers mainly involved in the expression of lipid synthesis-related enzyme genes. It is primarily expressed in the liver and adipocytes and regulates lipogenesis by changing its mRNA level (Shimano 2001). Previous research has shown that *SREBP-1c* can directly regulate the expression of many fat synthesis-related genes such as *ACC*, *FAS*, and *ME* (Gondret et al. 2001). Xie's test results show that curcumin can reduce the expression of *ACC*, *FAS*, and *SREBP-1c* genes, thereby reducing lipid deposition in broilers (Xie et al. 2019). This experiment showed that curcumin addition could include a linear reduction the expression of *ACC*, *FAS*, *ME*, *SREBP-1c* genes during 29–36 wk. These results indicated that curcumin supplementation inhibits fatty acid synthesis by lowering mRNA expression of genes involved in fatty acid synthesis, thereby lowering lipid build-up in the liver and belly of Roman layers and improving animal health. Triglycerides are transported mainly via VLDL. APOB-100 and AOPVLDLII are the primary components of a new VLDL that develops in the liver during egg production (Walzem et al. 1999). Special VLDL connects to receptor proteins on the follicle via APOB, enters the follicle by endocytosis, and supplies energy for the yolk's growth (Bujo et al. 1997; Walzem et al. 1999). According to this study, curcumin therapy also increased the mRNA expression of the APOB-100 and APOVLDLII. Up-regulation of these genes could be one of the factors ensuring the energy supply of the liver's synthesised fat to the follicle cells.

Conclusions

This study revealed that curcumin supplementation increased antioxidant capacity, decreased plasma and liver lipid profiles, decreased the activities of ME and LPL, decreased the mRNA expression levels of *ACC*, *FAS*, *SREBP-1c* and *ME*, and increased the mRNA expression levels of *APOB-100* and *APOVLDLII*. Our data suggested that the optimum concentration of curcumin was 150 or 250 mg/kg in the basal diets during 9 wk feeding trial.

Disclosure statement

The authors declare that they have no conflict of interest.

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Data availability statement

Data sharing does not apply to this article.

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