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EFFECTS OF DIETARY SUPPLEMENTATION WITH TAURINE ON PRODUCTION PERFORMANCE OF ANGORA RABBITS

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Abstract: This study aimed to evaluate the effects of dietary supplementation with taurine on production performance, serum biochemistry, immunoglobulin, antioxidant and hormones of Angora rabbits. A total of 160 8-month-old Angora rabbits with similar body weight were randomly assigned to one of four dietary groups, with 40 animals per group. The dietary groups consisted of the following different taurine supplementation levels: 0 (control), 0.1, 0.2, and 0.3% (air-dry basis). The 73-d feeding trial (from July 31 to October 11, 2016 in China) included a 7-d adjustment period and a 66-d experimental period. The results showed that taurine dietary supplementation had effects on feed consumption, hair follicle density and wool yield of the Angora rabbits ($P<0.05$), and adding 0.2% taurine could improve the wool yield. Compared with the control group, serum total cholesterol and low-density lipoprotein levels in supplemented groups were decreased ($P<0.05$). Dietary supplementation with taurine could improve the activity of superoxide dismutase, enhance total antioxidant capacity and reduce the content of malondialdehyde in serum ($P<0.05$). Besides, the serum level of thyroid (T4) hormone and insulin-like growth factor-1 in experimental groups was higher than that in the control group ($P<0.05$). In conclusion, taurine dietary supplementation could reduce the lipid metabolism, enhance the antioxidant capacity and hormone level of Angora rabbits, and adding 0.2% taurine could achieve the effect of increasing wool production.

Key Words: taurine, production performance, blood index, Angora rabbit.

INTRODUCTION

Angora rabbits represent valuable resources for the wool industry. Angora wool, which belongs to the luxury animal fibre category, is one of the most highly produced animal-hair fibres, after sheep wool and mohair. China is the largest producer of Angora wool, exporting 92% of global rabbit wool (Schlink and Liu, 2003). The wool production of Angora rabbits is affected by nutrition, genetics and other factors (Thébault *et al.,* 1992; Allain *et al.,* 1999; Katoch *et al.,* 1999; Rafat *et al.,* 2007; Bai *et al.*, 2019). Taurine, a sulphur-containing amino acid, is an important amino acid derivative that is abundantly distributed in many mammalian tissues (Lee *et al.,* 2004). Taurine has been found mainly in free form by experts, and is not used for protein synthesis (Bouckenooghe *et al.,* 2006). Previously, taurine had been considered an end product of sulphur amino acids metabolism with no biological significance, except its conjugation with bile acids to form bile salts that are essential for fat digestion. Subsequently, taurine was proven to play an important role in membrane stabilisation, anti-oxidation, detoxification, osmoregulation, growth modulation, calcium homeostasis and immunomodulation, as well as development of the neural retinal system (Wright *et al.,* 1986; Huxtable, 1992; Tdolini *et al.,* 1995; Pasantes-Morales *et al.,* 1998; Redmond *et al.,* 1998; Lima *et al.,* 2001; Militante and Lombardini, 2002; Lima *et al.,* 2004). Furthermore, taurine had also been implicated in the metabolism of proteins, lipid, minerals, glucose and cholesterol (Thompson and Tomas, 1987; Yun *et al.,* 2012; Zeng *et al.,* 2012). Huang and Peng (2008) reported that dietary taurine supplementation significantly improved the growth performance

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of piglets fed with a high plant protein diet. Adding 0.5 g/kg taurine to quail diet significantly increased taurine in the serum and egg yolk, significantly improved egg production and feed efficiency, reduced egg yolk cholesterol concentration and serum triglyceride level and did not affect egg quality (Wang *et al.,* 2010a, 2010b). Yannis *et al.* (2019) reported findings that 1.0% taurine supplementation in diets with high levels of soybean products might have a beneficial effect on growth performance and a pronounced effect on flesh quality of European sea bass. However, there has been limited information on the effectiveness of using taurine in rabbit diets in recent years. The aim of this study was to assess the use of taurine in the diets of Angora rabbits by examining its effects on production performance, biochemistry, immunoglobulin, antioxidants and hormones in serum.

MATERIALS AND METHODS

Ethics approval

The whole procedure for experimental animals was performed in strict accordance with guideline (IACC20160205) of the Institutional Animal Care and Use Committee of the Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences and performed following the Guidelines for Experimental Animals of the Ministry of Science and Technology (Beijing, China) for the protection of animals used for scientific purposes.

Animals and experimental design

In this study, a total of 160 8-month-old Angora rabbits with similar body weights (3415±300 g) were randomly assigned to one of four dietary groups, with 40 animals per group (20 males and 20 females). The dietary groups consisted of the following different taurine supplementation levels: 0 (control), 0.1, 0.2, and 0.3% (air-dry basis). The 73-d feeding trial (from July 31 to October 11, 2016 in China) included a 7-d adjustment period and a 66-d experimental period. The basic diet (Table 1) used in this study was formulated to meet the recommended nutrient requirements of growing rabbits (NRC, 1977). The experimental rabbits were housed singly in a cage (60×40×40 cm) and had *ad libitum* access to food and water. The animals were kept in a semi-controlled closed building during the experimental period at 18 to 25°C.

Sample collection and preparation

At the end of the trial, 32 rabbits (8 rabbits per treatment, 4 males and 4 females, with their body weights around the average group body weight) were used for blood sample collection from ear vein at 8:00 to 9:00 in the morning.

Table 1: Ingredients and chemical composition of the rabbit diet (%, air-dry basis).

Ingredients	%	Analysed composition [2]	%
Corn bran	12.3	Digestible energy (MJ/kg)	9.84
Soybean hull	13.3	Crude protein	17.00
Wheat bran	13.3	Crude fibre	16.60
Corn germ meal	12.3	Ether extract	3.40
Soybean meal	16.7	Crude ash	8.98
Peanut bran	5.4	Calcium	0.77
Rice hull	8.4	Total phosphorus	0.46
Soybean straw powder	5.5	Lysine	1.27
Artemisia argyi powder	8.3	Methionine+ Cystine	0.89
Soybean oil	0.5	Taurine (g/kg)	0.04
Premix [1]	4.0		
Total	100.0		

[1] The premix provided the following per kg diet, vitamin A: 10000 IU; vitamin D_3: 2000 IU; vitamin E: 50 mg; vitamin K_3: 2.5 mg; vitamin B_1: 5 mg; vitamin B_2: 10 mg; nicotinic acid: 20 mg, pantothenic acid: 50 mg; folic acid: 2.5 mg; vitamin B_{12}: 1 mg; choline chloride: 400 mg; Fe: 100 mg; Zn: 50 mg; Cu: 40 mg; Mn: 30 mg; I: 0.5 mg; Se: 0.05 mg; $CaHPO_4$: 15000 mg; NaCl: 5000 mg; Lysine: 1500 mg; Methionine: 1500 mg; the rest is miscellaneous meal carrier complement.

[2] Digestible energy was calculated, whereas the others were measured values.

The blood samples were kept at room temperature for natural serum release, and the serum was collected by pipette and stored in 1.5 mL centrifuge tube at –20°C for further analysis. Simultaneously, the mid-back skin samples (size: 0.5×0.5 cm) were collected after 10% chloral hydrate was injected intravenously at a dose of 1 mL/kg through ear vein, then fixed with 4% paraformaldehyde, making a paraffin section for hair follicle density analysis.

Chemical analysis of experimental diets

The International Association of Official Analytical Chemists procedures (AOAC, 2005) were used to determine the content of dry matter (method 934.01), crude protein (method 954.01), ether extract (method 920.39), crude fibre (method 978.10) and ash (method 942.05) in feeds. Crude protein content (6.25×N) and ether extract were determined using a Kjeltec Auto 1030 Analyser and a Soxtec 1043, respectively (FOSS Tecator AB, Höganäs, Sweden). The mineral profile (Ca, P) of the diet was analysed by ICP-OES (Spectro Ciros Vision EOP) after microwave digestion (method 999.10). The lysine and sulphur amino acids levels in the feed were analysed using an automatic amino acid analyser (Basic L-8900, Japan). All dietary chemical analyses were performed in duplicate. Taurine levels in the diets were measured by liquid chromatography (Agilent, model: 1260) according to the national standard of China (GB/T 5009.169-2003), and levels were 0.04, 1.05, 2.03, and 3.04 g/kg (air-dry basis).

Measurements and analyses

Wool production performance

Individual weight of animals was measured at the beginning and end of the trial. The total feed intake (FI) and the feed/wool ratio (F/W) were then calculated. Fibre length at different body points (back, buttocks, neck and both sides of the body) were measured with a steel ruler, take the average value as the final wool length. Rabbit wool fineness was determined by fibre fineness meter (YG002C; Changzhou Shuanggu Dunda Electromechanical Technology Co., Ltd) at microphotograph (CYG-055DI; Shanghai Institute of Optical Instruments), taking 100 hairs from one rabbit for determination and taking the average value as the final wool fineness. The mid-back skin samples were fixed with 4% paraformaldehyde and then dehydrated through a graded alcohol series, embedded in paraffin, sectioned at a thickness of 5 μm transverse section of skin, and stained with haematoxylin and eosin. The total hair follicle density (including primary hair follicle and secondary hair follicle numbers) in the back skin were measured using Image J software on five slides for each sample at 100× magnification with a light microscope (Nikon, ECLIPSE 80i) as reference Zhu *et al.* (2019).

Serum biochemistry

A sequential multiple analyser (Hitachi 7020, Japan) was used to analyse serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) following the manual of commercial protocols (WAKO, Japan).

Serum immunoglobulin

Serum immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM) titres were measured by enzyme-linked immunosorbent assays (ELISA) kit (Shang Hai Lengton Bioscience Co., China) according to the manufacturer's instructions.

Serum antioxidant properties

Serum superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content were measured by kits following the instructions provided by Nanjing Jiancheng Biological engineering Institute (Nanjing, China) and tested on ultraviolet-visible spectrophotometer (UV-2450).

Serum hormone level

Serum thyroid hormone (T4), insulin-like growth factor 1 (IGF-1) and melatonin (MTL) were analysed using commercial Radioactive Immune Assay Kits supplied by Tianjin Jiuding Company (Tianjin, China), and radioactivity was determined in DFM-96 10 tubes in a Radioactive Immune gamma counter.

Statistical analyses

The data were analysed by ANOVA and Duncan's test using the SPSS Statistics 17.0. Data are presented as mean±standard deviation, and *P*-value<0.05 was considered significant.

RESULTS AND DISCUSSION

Effects of taurine dietary supplementation on production performance

The effects of dietary supplementation with taurine on production performance and wool quality are summarised in Table 2. Taurine dietary supplementation had effects on feed consumption, hair follicle density and wool yield of the Angora rabbits (*P*<0.05), and adding 0.2% taurine could achieve the effect of increasing wool production. However, there were no differences among the groups concerning F/W, wool length and wool fineness of Angora rabbits (*P*>0.05).

Taurine is considered to be an essential nutrient for several animal species, including the cat, certain dogs, the fox, some monkeys and the anteater (Schaffer *et al.,* 2014). Liu *et al.* (2014) reported that 0.3% taurine supplementation in diets had different degrees of beneficial effects on piglet growth but excessive (1.5 or 3%) taurine had adverse effects on growth performance, liver and intestinal health of piglets. Similar results were also described in olive flounder, red seabream, meagre and black seabream that fed on the taurine supplemented diets (Park *et al.,* 2002; Matsunari *et al.,* 2008; De Moura *et al.,* 2018; Tong *et al.,* 2019). However, inclusion of taurine in a balanced chicken diet does not affect the production performance of poultry, and it is generally accepted that commercial poultry production is associated with a range of stressful situations (Surai and Fisinin, 2016a, 2016b; Schaffer and Kim, 2018). Therefore, the different effects of dietary supplement with taurine on the production performance of livestock and poultry should be considered and further studied.

Effects of dietary supplement with taurine on serum biochemistry

Compared with the control group, serum TC and LDL levels in experimental groups were decreased (*P*<0.05). However, there were no differences among the groups concerning TG, HDL, ALP, ALT and AST in serum of Angora rabbits (*P*>0.05; Table 3).

ALT and AST, mainly present in the liver, are two important enzymes for the conversion between protein and carbohydrate. When hepatic cells are damaged or cell membrane permeability increases, ALT and AST in liver would permeate into the bloodstream and, consequently, the activities of these enzymes would be detected in serum increase, which goes along with the lesion degree of hepatic cells (He *et al.,* 2013). Liu *et al.* (2014) reported that AST levels in serum were higher in pigs fed 1.5 and 3% taurine than those fed 0.3% taurine, indicating the possible

Table 2: Effects of taurine dietary supplementation on production performance and wool quality of Angora rabbits.

Items	Control	0.1% Taurine	0.2% Taurine	0.3% Taurine	*P*-value
Production performance					
Initial body weight[1] (g)	3337±270	3436±274	3439±295	3448±276	0.2748
Final body weight[2] (g)	3857±342	4026±354	4017±336	4101±332	0.0750
Wool yield (g)	219.0±33.6[a]	232.4±23.8[ab]	236.7±23.7[b]	244.0±29.1[b]	0.0145
Feed consumption (g)	13604±859[a]	14352±1100[b]	14528±955[b]	14551±975[b]	0.0015
Feed/wool ratio	63.3±8.9	62.2±6.4	61.9±6.8	60.2±5.6	0.4599
Wool quality					
Wool length (cm)	4.66±0.30	4.52±0.29	4.49±0.45	4.64±0.47	0.7614
Wool fineness (µm)	15.62±1.19	15.46±0.78	15.55±0.90	15.10±0.54	0.5853
Hair follicle density[3] (count/mm^2)	39.37±3.23[a]	41.92±4.63[ab]	45.65±0.85[b]	53.20±2.19[c]	0.0027

[1]8-month-old Angora rabbits. [2]the 73-day feeding trial.[3]at the end of the trial, 32 rabbits (8 rabbits per treatment, 4 males and 4 females). Data were presented as mean±standard deviation.
[a,b,c]Means in the same row with different superscript are significantly different (*P*<0.05).

Table 3: Effects of taurine dietary supplementation on serum biochemistry of Angora rabbits[1].

Items	Control	0.1% Taurine	0.2% Taurine	0.3% Taurine	*P*-value
Total cholesterol (mmol/L)	1.35±0.30[b]	0.89±0.22[a]	0.98±0.24[a]	0.90±0.26[a]	0.0371
Triglyceride (mmol/L)	1.04±0.43	0.80±0.30	0.83±0.34	0.67±0.22	0.3804
High-density lipoprotein (mmol/L)	0.52±0.19	0.51±0.13	0.49±0.041	0.54±0.16	0.9574
Low-density lipoprotein (mmol/L)	0.57±0.18[b]	0.26±0.10[a]	0.33±0.15[a]	0.26±0.10[a]	0.0050
Alkaline phosphatase (U/L)	54.28±18.58	53.55±7.11	50.09±12.27	43.29±7.16	0.4629
Alanine aminotransferase (U/L)	21.99±2.00	29.59±10.92	26.40±4.50	24.24±3.88	0.3012
Aspartate aminotransferase (U/L)	81.48±4.76	108.3±37.17	97.26±11.9	82.73±7.01	0.1481

[1]At the end of the trial, 32 rabbits (8 rabbits per treatment, 4 males and 4 females). Data were presented as mean±standard deviation.
[a,b]Means in the same row with different superscript are significantly different ($P<0.05$).

damage in liver of pigs at high taurine levels. Furthermore, liver damage could be induced by higher oxidative stress, which always couples with higher serum AST and ALT levels (Chang *et al.,* 2010; Huang *et al.,* 2012). Interestingly, sixty-day 1% taurine supplementation in drinking water for hyperlipidaemia and atherosclerosis-prone Japanese quail fed a high-cholesterol (1%) diet significantly reduced serum non-HDL cholesterol and dramatically decreased the serum triglyceride level (Murakami *et al.,* 2010). Importantly, taurine could reduce the level of serum lipid peroxidation and improve the antioxidant capacity of broilers fed with taurine for one week (Huang *et al.,* 2014a, 2014b). Current research has shown that dietary supplement of taurine could reduce the TC and LDL in blood of Angora rabbits, which means that taurine plays an important role in lipid metabolism.

Effects of taurine dietary supplementation on serum immunoglobulin and antioxidant properties

The effects of taurine dietary supplementation on serum immunoglobulin and antioxidant properties are reported in Table 4. Dietary supplementation with taurine could improve the activity of SOD, enhance T-AOC and reduce the MDA content ($P<0.05$). On the contrary, the IgA, IgG and IgM indices were not improved after adding taurine to serum of Angora rabbits ($P>0.05$).

Taurine has been proven to be a powerful mediator of immune responses in mammals by regulating the proliferation of lymphocytes and prohibiting the secretion of pro-inflammatory cytokines (Salze and Davis, 2015). Furthermore, it has been reported that dietary taurine can induce the overexpression of immune-related genes (Cheng *et al.*, 2018), but down-regulate the mRNA abundance expressions of the antioxidant enzymes and inflammation genes (Zhang *et al.,*2018) that could consequently improve immune status in fish. However, the IgA, IgG and IgM indices in serum of Angora rabbits were not improved after adding taurine.

Many studies have shown that taurine can exert its antioxidant properties by quenching various radicals or by improving and/or restoring the antioxidant enzyme activities (Rosemberg *et al.,* 2010; Bañuelos Vargas *et al.,* 2014; Salze and Davis, 2015). Furthermore, taurine is not only a precursor of glutathione (Hayes *et al.,* 2011), but can also

Table 4: Effects of taurine dietary supplementation on serum immunoglobulin and antioxidant properties of Angora rabbits[1].

Items	Control	0.1% Taurine	0.2% Taurine	0.3% Taurine	*P*-value
Serum immunoglobulin					
Immunoglobulin A (g/L)	0.75±0.02	0.78±0.09	0.83±0.10	0.81±0.09	0.3928
Immunoglobulin G (g/L)	8.35±0.48	7.68±0.75	7.93±0.36	7.75±0.47	0.2374
Immunoglobulin M (g/L)	0.70±0.04	0.70±0.05	0.67±0.06	0.64±0.02	0.1621
Serum antioxidant properties					
Superoxide dismutase (U/mL)	77.38±3.21[a]	86.07±3.17[b]	88.75±2.57[b]	89.29±3.61[b]	<0.0001
Total antioxidant capacity (U/mL)	7.82±0.92[a]	8.49±0.90[ab]	8.54±0.47[ab]	8.88±0.36[b]	0.1606
Malondialdehyde (nmol/mL)	5.11±0.003[d]	4.86±0.006[c]	4.63±0.002[b]	4.54±0.002[a]	<0.0001

[1]At the end of the trial, 32 rabbits (8 rabbits per treatment, 4 males and 4 females). Data were presented as mean±standard deviation.
[a,b,c,d]Means in the same row with different superscript are significantly different ($P<0.05$).

Table 5: Effects of taurine dietary supplementation on serum hormones of Angora rabbits[1].

Items	Control	0.1% Taurine	0.2% Taurine	0.3% Taurine	*P*-value
Thyroid hormone (pg/mL)	25.67±1.20[a]	33.39±5.04[b]	34.98±7.41[b]	37.20±6.06[b]	0.0220
Insulin-like growth factor 1 (ng/mL)	168.20±10.79[a]	189.32±10.66[b]	206.64±13.67[b]	208.07±23.63[b]	0.0030
Melatonin (ng/mL)	294.01±11.50	298.82±24.62	301.12±22.65	314.14±13.54	0.4047

[1]At the end of the trial, 32 rabbits (8 rabbits per treatment, 4 males and 4 females). Data were presented as mean±standard deviation.
[a,b]Means in the same row with different superscript are significantly different ($P<0.05$).

enhance regeneration of glutathione from glutathione disulphide (Morales *et al.*, 2004; Pérez-Jiménez *et al.*, 2012; Bañuelos Vargas *et al.,* 2014). In addition, taurine can protect mitochondrial cell integrity and respiration and regulate protein synthesis in this organelle, which consequently augments the electron transport chain activity and protects the mitochondria from generation of superoxide radicals (Chian *et al.,* 2012). Roghayeh *et al.* (2020) reported that supplementing diets with crystalline taurine up to 12.5 g/kg significantly improved antioxidant enzyme activities and decreased the lipid peroxidation ratio in the liver, and 15.6 g/kg taurine level remarkably reduced the liver antioxidant activities and increased the MDA content, which could be due to possible disorders in hepatic glutathione synthesis as a consequence of the liver malfunction. In this study, we found that dietary supplementation with taurine could improve the activity of SOD, enhance T-AOC and reduce the content of MDA in serum, which were consistent with the above research results.

Effects of dietary supplementation with taurine on serum hormone level

From Table 5, it can be seen that the serum levels of T4 and IGF-1 in experimental groups were higher than in the control group ($P<0.05$). However, the MTL levels in serum were similar among the 4 groups ($P>0.05$).

Pituitary T4 is the principal hormone that regulates postnatal growth in animals. T4 can promote re-epithelialisation and angiogenesis in wounded human skin *ex vivo*. MTL is related to the seasonal growth of wool; after implantation of melatonin, it can promote the early maturation of fur (Allain and Rougeot,1980; Rougeot *et al.,*1986; Allain and Thébault, 1988). IGF-1 elicits extensive anabolic effects in various tissues, plasma concentrations of IGF-1 increase significantly with increasing nutrient intake, and the circulating IGF-1 concentrations are sensitive to nutritional changes (Smith *et al.,* 2002). Current research has shown that after adding taurine, the level of T4 hormone and IGF-1 in serum was higher than in the control group.

CONCLUSIONS

In conclusion, dietary supplementation with taurine could reduce the lipid metabolism, enhance the antioxidant capacity and serum hormone level of Angora rabbits, and adding 0.2% taurine could achieve the effect of increasing the wool yield.

Conflict of interest: We certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

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