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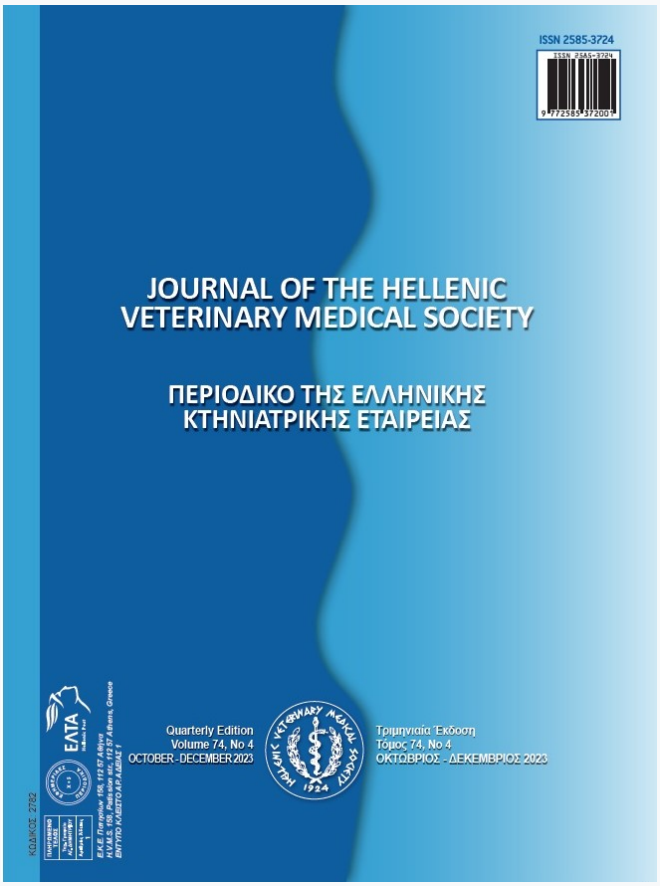

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A Sha, Y Liu, H Hao

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Research article
Ερευνητικό άρθρο

Effects of *Suaeda rigida* extract on the growth performance, antioxidant capacity and immune function of Karakul sheep

Ailong Sha[1], Yi Liu[2], Haiyan Hao[3]

[1]*School of Teacher Education, Chongqing Three Gorges University, Chongqing, China*

[2]*School of biology and food engineering, Chongqing Three Gorges University, Chongqing, China*

[3]*School of Environmental and Chemical Engineering, Chongqing Three Gorges University, Chongqing, China*

ABSTRACT: The objectives of this study were to investigate the effects of dietary supplementation of *Suaeda rigida* extract on the growth performance, antioxidant capacity and immune function of Karakul sheep. Blood samples were collected from the jugular veins before morning feeding on the 10th, 20th and 30th days of the trial period, respectively, and the serum antioxidant and immune indexes of Karakul sheep in the control and experimental groups were determined. The results showed that the growth performance of Karakul sheep was dramatically improved by dietary supplementation of *Suaeda rigida*, the contents of NO, cGMP, MDA, H_2O_2, •OH and the activities of XOD, GGT, NOS in the serums of Karakul sheep were inhibited to varying degrees, the GSH content and the activities of GSH-Px, GST, CAT and SOD were substantially increased ($P<0.05$ at 10d, $P<0.01$ both 20d and 30d). At 30 days, the contents of total protein, globulin, calcium and three immunoglobulins including IgG, IgA, IgM in the sera of experimental Karakul sheep were significantly higher than those in the control group ($P<0.05$). All the results suggested that the growth performance, antioxidant, anti-aging capacity, immune function, and life extension of Karakul sheep were significantly improved by dietary supplementation with *Suaeda rigida*.

Keywords: *Suaeda rigida*, growth performance, oxygen radical, NO, glutathione, immune

Corresponding Author:
Ailong Sha, School of Teacher Education, Chongqing Three Gorges University, 404100, Wanzhou, Chongqing, P.R. China
E-mail address: lyshaailong@163.com

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INTRODUCTION

Suaeda rigida Kung et G. L.Chu, the tallest dicotyledonous plant in *Suaeda*, Chenopodiaceae, Caryophyllales, is endemic to the Tarim Basin (Feng *et al*., 2003). It is mainly distributed in the Aksu-Bachu area of Xinjiang, with strong vitality, large biomass and high nutrient content. Local residents often collect its young leaves for edible wild vegetables (Sha and Hao, 2021). Because of its narrow distribution, the low population density and few scientific research institutes and universities in its distribution area, there are few reports on *Suaeda rigida* at home and abroad. The chemical constituents and pharmacological effects of *Suaeda rigida* were studied in the early stage of our research group. The results showed that *Suaeda rigida* contains polysaccharides, alkaloids, volatile oils, flavones, saponins, and other chemical components (Hao and Sha, 2013), which can inhibit intestinal contraction of animals (Sha and Hao, 2021), protect normal tissue cells and other pharmacological effects (Sha *et al*., 2013). In order to rationally utilize the resources of *Suaeda rigida* and further study its medicinal and feeding value, Karakul sheep, which are mainly distributed in southern Xinjiang of China, were selected in this experiment to investigate their growth performance, antioxidant capacity and immune function by dietary supplementation of *Suaeda rigida* extract. The purpose of this study is to choose an effective Chinese herbal medicine feed additive and forage for Karakul Sheep's growth and development, immune function, anti-oxidation, anti-aging, metabolism and health regulation.

MATERIALS AND METHODS

Preparation of *Suaeda rigida* extract

Suaeda rigida was collected from the 12th regiment of the 1st Division and identified by haoping Yang, Department of Botany, Tarim University, crushed after drying in the shade. An appropriate amount of *Suaeda rigida* powder was weighed, 10 times the volume of 95% ethanol was added, and it was refluxed and extracted in a water bath at 85℃ for 2 h. The filtrate was collected (the residue was repeatedly extracted for 3 times). The extracts were combined, evaporated and concentrated to a paste, and then dried in an oven at 75℃ to obtain the ethanol extract of *Suaeda rigida*, which contained 40% polysaccharides, 34% alkaloids, 12% flavonoids, 6% volatile oil and grease, 4% saponins and steroidal saponinsand, and 4% total phenolic.

Experimental animals and grouping

Eighteen Karakul sheep with good body condition and similar body mass (mean body mass 39.36 ± 1.06 kg) were randomly divided into control and test groups, with 9 animals in each group (female equal male) and no substantial difference in initial body mass between the two groups (P>0.05). The basal diet was fed during the trial feeding period (the ratio of concentrate to roughage was 1:1, as shown in Table 1). During the test period, *Suaeda rigida* extract (20 g/kg basal diet) was added to the roughage of the basic diet of Karakul sheep in the test group, but not in the control group. The care and use of sheep were approved by the Scientific Ethics Committee for Experiments on Animals of the Tarim University. All applicable international, national, and institutional guidelines for the care and use of animals were followed, for example, the Chinese Guidelines for the Care and Use of Animals for Research (GB 14925, 2001).

Chemicals and Reagents

Commercial kits used for the determination of nitric oxide (NO), inducible nitric oxide synthase

Table 1. Composition and nutrient levels of basal diets (air-dried basis) g/kg

Ingredients	Content	Nutrient levels	
Cottonseed hull	250.0	Total energy/ (MJ/kg)	18.16
Straw	250.0	Net energy/ (MJ/kg)	10.49
Bran	83.1	Dry matter	898.1
Soybean meal	65.3	Crude protein	132.0
Cottonseed meal	43.0	Organics	865.3
Corn	288.1	Acid detergent fiber	87.2
NaCl	5.5	Neutral detergent fiber	307.4
Urea	4.5	Ca	8.6
Premix	10.5	P	6.5

Note: The premix provided the following per kg of diets: Cu ($CuSO_4$•$5H_2O$) 4.4 g, Fe ($FeSO_4$•$7H_2O$) 18.8 g, Mn ($MnSO_4$•$4H_2O$) 9.2 g, Zn ($ZnSO_4$•$7H_2O$) 11.6 g, Se (Na_2SeO_3) 72 mg, I (KI) 140 mg, Co ($CoCl$•$6H_2O$) 80 mg, VA 100 000 IU, VD 50 000 IU, VE 1 000 IU.

(iNOS), total nitric oxide synthase (TNOS), cyclic guanosine monophosphate (cGMP), coomassie blue protein, glutathione-s transferase (GST), glutathione peroxidase (GSH-Px), trace reduced glutathione (GSH) (microplate assay, 96T), catalase (CAT), superoxide dismutase (SOD), xanthine oxidase (XOD), malondialdehyde (MDA), hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), and ELISA kits for immunoglobulin A (IgA), G (IgG), M (IgM) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). $Na_2HPO_4 \cdot 12H_2O$, KH_2PO_4 and NaCl were analytical pure, obtained from Sinopharm Chemical Reagent Co., Ltd., China.

Feeding Management

The experiment was carried out in the sheep breeding farm of the animal experiment station of Tarim University. Before the experiment, the sheep farm was cleaned and disinfected. After drying, the trial sheep were weighed, numbered and grouped, and then deworming and related epidemic prevention work were carried out. The management conditions were consistent. All trial sheep were fed the same basal diet (without the addition of *Suaeda rigida* extract) for 7 days, and the behavior and health status of the sheep were observed for the timely adjustment. During the 30 days of the test period, the trial sheep were individually housed, and they were all fed daily with coarse and then fine separately at 8:00 and 20:00, with the free feeding and drinking. Each sheep was weighed on the platform scale at 09:00 on the 0th day and 31st day of the test period, and the feed intake of each sheep was recorded every day.

Determination index and method

Determination of growth performance

Daily increment: the weight gained by each trial sheep during the trial divided by the number of days.

Daily feed intake: the daily feed intake is the difference between the feed supply and the surplus of each experimental sheep. Average daily gain and average daily feed intake were then calculated based on the number of tested sheep per group.

Feed to Gain ratio: the ratio of the amount of feed consumed to the body weight gain of each sheep during the trial.

Blood sampling, serum preparation and serological analysis

Blood samples were collected from the jugular veins of two groups of Karakul sheep before morning feeding on the 10th, 20th and 30th days of the trial period. They were placed in a centrifuge tube without anticoagulant until natural coagulation. After centrifugation at 3000 r/min for 20 min, the serums were separated and determined rapidly.

Determination of serum antioxidant indexes

The contents of NO, cGMP and the activities of iNOS, TNOS were determined by colorimetric method according to the kit specification provided by Nanjing Jianceng Institute of Biological Engineering (Nanjing, China). The structural nitric oxide synthase (cNOS) activity equals the activity (TNOS minus iNOS). The activities of GST, GSH-Px, SOD, CAT, XOD and the contents of GSH, MDA, •OH, H_2O_2, protein were measured according to the kit instructions provided by Nanjing Jiancheng Bioengineering Institute using UV-Vis (Shanghai Lengguang Technology Co., Ltd., China) and an iMark Microplate Reader (Bio-Rad, USA). Glutamyl aminotransferase (GGT) was determined by Vitros 350 automatic dry emergency rapid biochemistry analyzer (Johnson Co., USA).

Determination of serum immune indexes

IgG, IgA,and IgM were measured by enzyme-linked immunoassay (ELISA), all the kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and the specific procedures were performed according to the respective kit instructions by an iMark Microplate Reader (Bio-Rad, USA). The total protein, globulin, white globulin ratio, calcium (Ca) and magnesium (Mg) were determined by Vitros 350 automatic dry emergency rapid biochemistry analyzer (Johnson Co., USA).

Statistical processing

Data were processed using SPSS23.0 statistical software, each index of different treatments was F-tested by one-way ANOVA and those with significant differences were analyzed by Duncan's method for multiple comparisons. The results were presented as mean ± standard deviation (± s).

RESULTS

Effects of dietary supplementation of *Suaeda rigida* extract on growth performance of Karakul Sheep

It can be seen from Figure 1 that the average daily gain of Karakul sheep in the experimental group (252.50±20.79) was significantly higher than that of

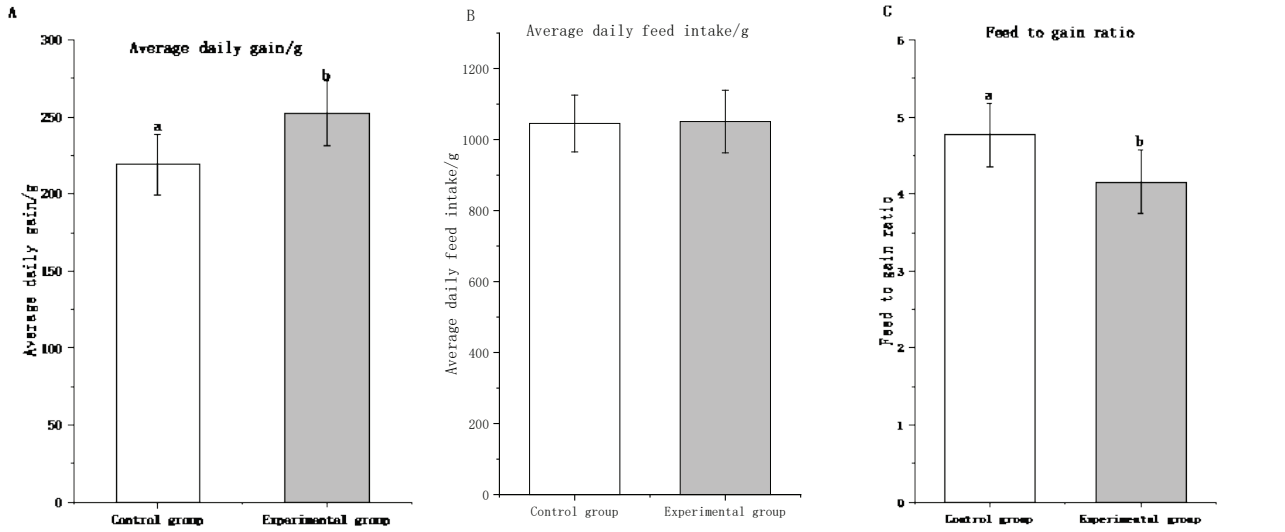

Figure 1. Effects of *Suaeda rigida* extract on the Growth Performance of Karakul sheep

Note: Compared with the control group, with different small letter superscripts mean significant difference (P<0.05), values with no letter mean no significant difference (P>0.05).

Table 2. Effects of *Suaeda rigida* extract on NOS-NO-cGMP signal pathway in serum of Karakul Sheep ($\bar{x}$±s)

Items	Grouping	10d	20d	30d
NO (umol/L)	Control group	344.52±54.95^{a}	472.78±82.50^{b}	491.83±34.06
	Experimental group	240.46±30.05^{*a}	289.92±30.17^{**b}	335.26±21.61^{**c}
TNOS (U/mL)	Control group	18.16±0.91^{A}	27.43±1.24^{Bb}	30.50±2.52^{c}
	Experimental group	11.59±1.40^{**A}	17.21±1.14^{**B}	22.87±1.23^{**C}
cNOS (U/mL)	Control group	11.79±1.46^{A}	16.58±0.51^{B}	19.32±0.93^{C}
	Experimental group	9.12±0.62^{*A}	12.29±1.01^{**B}	14.93±1.15^{**C}
cGMP (ug/L)	Control group	16.22±2.48^{A}	24.87±4.23^{B}	25.95±3.57
	Experimental group	12.47±1.71^{*a}	15.63±2.59^{**b}	15.96±2.25**

Note: In the same column, the same indicators of the experimental group and control group, $^{**}P$<0.01, means highly significant difference, $^{*}P$<0.05, means significant difference. In the same row, while with different capital letter superscripts mean very significant difference (P<0.01), with different lowercase letter superscripts mean significant difference (P<0.05), and no letter superscripts mean no significant difference (P>0.05). The same as table 4 and 5 below.

the control group (219.17±19.56) at 30 days, and the feed to gain ratio (4.16 ±0.42) was significantly lower than that of the control group (4.77 ±0.41) (P<0.05). Compared with the control group (1045.32±80.07), the average daily feed intake of Karakul sheep in the experimental group (1050.68±88.03) was increased, but the difference was not significant (P>0.05).

Effects of dietary supplementation of *Suaeda rigida* extract on antioxidant capacity of Karakul Sheep

Effects of *Suaeda rigida* extract on NOS-NO-cGMP signal pathway in Karakul sheep serum

As shown in Table 2, the contents of NO and cGMP and the activities of TNOS and cNOS in the serum of Karakul sheep of the experimental group were substantially lower than those of the control group. Among them, the contents of NO, cGMP and the activity of cNOS were significantly lower than those of the control group at 10d (P<0.05), and the activity of TNOS was very significantly reduced (P<0.01). The contents of NO, cGMP and the activities of TNOS and cNOS at 20d and 30d were considerably different from those in the control group (P<0.01). NO, cGMP, TNOS and cNOS in both the experimental group and the control group were increased gradually with the increase of time, especially at 20d than at 10d (GMP, TNOS and cNOS P<0.01, NO P<0.05).

Effects of *Suaeda rigida* extract on GSH content and related enzymes activities in serum of Karakul Sheep

As shown in Table 3, the serum GSH content and the activities of GSH-Px and GST in Karakul sheep of the experimental group were considerably increased relative to the control group. Among them, the GSH

Table 3. Effects of *Suaeda rigida* extract on the GSH content and related enzymes activities in the Serum of Karakul Sheep (x±s)

Items	Grouping	10d	20d	30d
GSH (mg/g proton)	Control group	88.49±3.35	89.86±5.07	92.36±8.86
	Experimental group	95.97±2.88^{*A}	140.26±12.50^{**B}	152.48±17.31**
GSH-P_X (U)	Control group	194.11±57.33	218.32±37.41	227.91±8.52
	Experimental group	301.45±9.93^{*A}	437.84±65.28^{**B}	297.43±16.17^{**C}
GST (U/mg proton)	Control group	9.30±0.73	9.62±0.43^{A}	12.33±1.30^{B}
	Experimental group	9.42±0.33^{A}	16.08±0.45^{**B}	24.73±4.55^{**C}
GGT/ (U/L)	Control group	66.48±8.73	65.00±9.46	62.50±7.87
	Experimental group	60.47±7.20	57.36±7.29	55.08±8.94

Table 4. Effects of *Suaeda rigida* extract on Oxygen Free Radicals and Related Enzymes activities in Karakul sheep serum (x±s)

Items	Grouping	10d	20d	30d
MDA (nmol/ml)	Control group	4.77±0.65	4.39±0.38	4.69±0.34
	Experimental group	4.56±0.33^{A}	2.87±0.41$^{** B}$	2.49±0.31**
H_2O_2(mmol/gprot)	Control group	1.20±0.12^{a}	1.42±0.11^{b}	1.47±0.31
	Experimental group	0.85±0.01**	0.88±0.09$^{** a}$	0.99±0.05$^{* b}$
•OH (one)	Control group	28.39±4.36	28.43±3.91	27.58±2.22
	Experimental group	21.16±3.42*	20.12±2.61**	19.64±3.54**
XOD(U/L)	Control group	11.47±1.50^{A}	5.77±0.92^{B}	8.18±0.08^{C}
	Experimental group	10.47±2.02^{A}	5.53±0.18^{B}	7.71±0.81^{C}
SOD (U/ml)	Control group	92.88±6.44	95.12±10.52	94.63±9.48
	Experimental group	102.09±6.46*	109.57±4.00$^{* A}$	121.23±3.94$^{** B}$
CAT (U/ml)	Control group	0.46±0.09^{A}	0.15±0.04^{B}	0.46±0.11^{C}
	Experimental group	0.64±0.12	0.49±0.21$^{* a}$	0.75±0.09$^{** b}$

content and GSH-Px activity were substantially higher than those of the control group at 10 days (P<0.05), but the GST activity was not considerable (P>0.05); the three formers at 20 and 30 days were significantly different from those in the control group (P<0.01). The activities of GSH-Px and GST in serum of Karakul sheep of the experimental group were quite different from those of 10 days and 20 days and those of 20 days and 30 days (P<0.01). The GSH content of Karakul sheep in the experimental group was substantially different between 10 days and 20 days (P<0.01). The GST activity in the serum of Karakul sheep in the control group was considerably different between 20d and 30d (P<0.01). Compared with the control group, the GGT activity in the Karakul sheep serum of the test group was reduced in the three measurements, but the differences were not significant (P>0.05).

Effects of *Suaeda rigida* extract on Oxygen Free Radicals and Related Enzymes activities in Karakul sheep serum

As shown in Table 4, the contents of MDA, H_2O_2 and •OH in serum of Karakul sheep of the experimental group were substantially decreased relative to the control group, the activity of XOD was decreased, but the difference was not significant (P>0.05). At 10 days, the MDA content was decreased not significantly (P>0.05), the •OH content significant (P<0.05), and the H_2O_2 content very significant (P<0.01); and the three reductions were all extremely significant at 20 days (P<0.01); At 30 days, the contents of MDA, •OH and H_2O_2 were significantly decreased (including MDA and •OH P<0.01, H_2O_2 P<0.05). The activities of SOD and CAT in the serum of Karakul sheep in the experimental group were considerably higher than those in the control group. Among them, SOD activity was increased significantly at 10 days (P<0.05), CAT activity had no substantial change (P>0.05). Both of them were significantly increased at 20d (P<0.05), and very considerable at 30d (P<0.01).

Effects of *Suaeda rigida* extract supplementation on immune function of Karakul sheep

As shown in Table 5 that compared with the control group, the content of magnesium in the serum of Karakul sheep in the experimental group was decreased, but the difference was not considerable (P>0.05). At 30 days, the contents of total protein, globulin, IgG, IgA and IgM in the serum of Karakul sheep in the experimental group were substantially higher than those in the control group (P<0.05); However, at 10d and 20d, the above 5 indexes in the serum of experimen-

Table 5. Effects of *Suaeda rigida* extract on hematological and biochemical parameters of Karakul Sheep (***x*±s**)

Item	Control group			Experimental group		
	10 d	20 d	30 d	10 d	20 d	30 d
TP/ (g/L) Total protein	67.27±6.31	67.10±5.85	66.80±5.96^{a}	71.08±4.93	69.72±4.60	76.94±6.53^{b}
GLB/ (g/L) Globulin	38.06±4.19	38.10±4.65	38.00±5.20^{a}	40.97±3.84	41.50±3.76	46.00±4.50^{b}
IgG/ (mg/mL)	29.36±3.25	29.43±3.29	29.18±4.03^{a}	31.25±2.97	32.17±3.52	35.84±3.71^{b}
IgA/ (mg/mL)	4.53±0.51	4.56±0.50	4.48±0.47^{a}	4.85±0.52	4.97±0.55	5.75±0.80^{b}
IgM/ (mg/mL)	2.42±0.30	2.45±0.28	2.43±0.33^{a}	2.75±0.34	2.83±0.35	3.17±0.42^{b}
A/G White globulin ratio	0.75±0.07	0.76±0.06	0.76 ±0.07^{a}	0.70±0.05	0.69±0.04	0.67±0.04^{b}
Ca/ (m mol/L) Calcium	2.08±0.10^{A}	2.10±0.13^{A}	2.01±0.11^{A}	2.30±0.05^{B}	2.34±0.05^{B}	2.29±0.07^{B}
Mg/ (m mol/L) Magnesium	1.61±0.22	1.63±0.16	1.60±0.07	1.54±0.12	1.56±0.23	1.53±0.12

Note: In the same measurement time, the same indicators of the experimental group and control group, while with different small letter superscripts mean significant difference (P<0.05), and with different capital letter superscripts mean significant difference (P>0.01), values with no letter mean no significant difference (P>0.05). In the different measurement times, the same indicators of the experimental group or control group, the difference between a * indicates significant difference (P<0.05), the difference between the two * indicates very significant difference (P<0.01), and is not marked * shows no significant difference (P>0.05).

tal group were increased, but the differences were not considerable (P>0.05). At 30d, the white globulin ratio in the serum of Karakul sheep in the experimental group was significantly lower than that in the control group (P<0.05), at 10d and 20d, it was decreased but the difference was not substantial (P>0.05). Compared with the control group, the serum calcium content of Karakul sheep in the experimental group was very substantially increased during the three determinations (P<0.01).

DISCUSSION

Effects of dietary supplementation of *Suaeda rigida* extract on growth performance of Karakul Sheep

The results showed that the dietary supplementation of *Suaeda rigida* extract can significantly increase the average daily gain of Karakul sheep, the feed to gain ratio was substantially lower than that of the control group, and the average daily feed intake was increased. It fully showed that *Suaeda rigida* extract could dramatically improve the growth performance of Karakul sheep. *Suaeda rigida* is an excellent pasture resource in Tarim Basin, and fresh *Suaeda rigida*, Karakul sheep like to eat. In this experiment, *Suaeda rigida* extract was added to the crude diet, which may affect the taste of sheep, so that the average daily feed intake of the experimental group was increased slightly compared with the control group, but there was no substantial difference. If fresh *Suaeda rigida* can be fed, the daily feed intake of sheep may be improved, which needs to be confirmed by further studies. Previous studies have shown that other *Suaeda* plants contain a large amount of nutrients (Li *et al*., 2019; Wang *et al*., 2014; Zheng *et al*., 2011), but there has been no report on the nutritional composition of *Suaeda rigida*. Whether *Suaeda rigida* extract contains nutrients needed for the growth of Karakul sheep needs to be confirmed by further studies.

Effects of dietary supplementation of *Suaeda rigida* extract on antioxidant capacity of Karakul Sheep

Effects of *Suaeda rigida* extract on NOS-NO-cGMP signal pathway in Karakul sheep serum

There are unpaired electrons in the molecular structure of NO, which is a free radical, which plays a dual role in physiology and pathology in animals. On the one hand, it can inhibit and kill viruses and other microorganisms, and participate in the body's anti-infection immunity and defense; On the other hand, a large dose of NO can produce cytotoxic effects and damage normal tissue cells (it can produce peroxide nitrite with O_2•- and other active nitrogen with O_2, thus causing damage to the body, and can also enhance oxidative damage caused by H_2O_2 and LOO•) (Bedenbaugh *et al*., 2018). Endogenous NO is produced when nitric oxide synthase (NOS) catalyzes the formation of guanine from L-arginine (L-Arg) (Berlinguer *et al*., 2020). According to the tissue source,

NOS can be divided into endothelial type (eNOS), neural type (nNOS) and inducible type (iNOS). eNOS and nNOS are also called Structural NOS (cNOS). Studies have shown that cytokines and active peptides in vivo, polysaccharides and other active substances in vitro can regulate the immune defense of animals through NOS and eventually through NO. The action target of NO is inactive guanylate cyclase (GC). NO can change the conformation of GC by combining with Fe^{2+} in hemoglobin, and activate GC in the form of Nitrosyl-hemoglobin - enzyme protein complex, and promote the transformation of guanosine triphosphate (GTP) into cyclic guanosine phosphate (cGMP) (Ignarro *et al*., 1982). cGMP can further induce NO production through the signal cascade effect.

The results showed that *Suaeda rigida* extract could inhibit the contents of NO and cGMP and the activities of TNOS and cNOS in the serum of Karakul sheep. The contents of NO and cGMP and the NOS activity in serum of Karakul sheep were significantly inhibited by dietary addition of *Suaeda rigida* extract at 10d ($P<0.05$), and the inhibitions were highly significant at 20d and 30d ($P<0.01$), and the inhibition became more and more obvious with the increase of adding time. NO, cGMP, TNOS and cNOS in both the control and experimental groups were increased gradually with the increase of time, especially at 20d, which were more evident than those at 10d. The reason may be that the higher and higher temperature at 10d (5-26), 20d (6-5) and 30d (6-15) resulted in the NOS activity higher and higher (Ewing the temperature rose abruptly in June), and thus the NO content was increased more and more. The increase of temperature accelerated the enzymatic reaction and caused excessive accumulation of NO in sheep, which caused toxic damage to normal tissue cells, and accelerated the oxidation and senescence process of sheep. The addition of *Suaeda rigida* extract can delay the oxidation and senescence of sheep and prolong their life.

Studies have shown that *Suaeda* plants contain polysaccharides, flavonoids, vitamins, trace elements and other chemical components (Wang *et al*., 2014), all of which have the effects of anti-oxidation (Wang *et al*., 2018) and anti-aging (Zhang *et al*., 2013). *Suaeda rigida* extract inhibited NO, cGMP, NOS, and anti-oxidative senescence, indicating that *Suaeda rigida* may contain the components mentioned above, which is consistent with our previous research results (Hao and Sha, 2013).

Effect of *Suaeda rigida* extract on the GSH content and related enzymes activities in serum of Karakul Sheep

Glutathione is the most important small molecule active oligopeptides in the antioxidant defense system in vivo. It can be divided into oxidized form (GSSG) and reduced form (GSH), and mainly exists as GSH under physiological conditions. As the substrate of GSH-Px or GST, GSH can scavenge harmful free radicals (primarily oxygen free radicals) or lipid peroxides in organisms and convert them into fatty acids and water. At the same time, GSH is oxidized to GSSG (Kasidit *et al*., 2021). GSH can stabilize sulfhydryl enzymes and prevent hemoglobin and other cofactors from oxidative damage. Therefore, the amount of GSH and the activities of its substrate GSH-Px and GST are essential factors to measure the antioxidant capacity of the organism. GGT mainly exists in liver cell membrane and microsomes, and participates in the metabolism of GSH, GGT in serum mainly comes from the hepatobiliary system (Corti *et al*., 2017). The study found that the content of GSH and the activities of GSH-Px and GST in the serum of Karakul sheep can be significantly increased by adding an appropriate amount of *Suaeda rigida* extract, and the activity of GGT in its serum can be reduced, indicating that *Suaeda rigida* extract can enhance the ability to Scavenge free radicals and protect the liver of Karakul sheep.

In recent years, only one of glutathione and its related enzymes has been studied in studies on the effects of Chinese herbal feed additives on the antioxidant function of sheep. For example, the research results of Zhang *et al.* (2021) found that rosemary extract could significantly improve the GSH-Px activity in the serum of dairy goats. The research results of Cai *et al.* (2021) showed that the activity of GSH-Px in the betaine-supplemented treatment groups significantly increased relative to the control group. Sa *et al.* (2020) showed that dietary addition of *Gymnadenia conopsea* polysaccharides could first decrease and then increase the GSH-Px activity in the serum of sheep under oxidative stress. The change of a single index can only reflect the influence of the test object on its existence, there is a particular contingency, which is not enough to reflect the change of the actual antioxidant capacity of animals. In this study, GSH, GSH-Px, GST and GGT, which interact with each other and are closely related in the antioxidant system, were studied as a system to avoid the accident of the experiment and improve the accuracy of the in-

vestigation. The effects of *Suaeda rigida* extract supplementation on the serum GSH and related enzymes activities of Karakul sheep were evaluated comprehensively and objectively.

Effects of *Suaeda rigida* extract on Oxygen Free Radicals and Related Enzymes activities in Karakul sheep serum

The production and elimination of free radicals in animals have always been in a dynamic balance, when the body is stimulated by external pathogenic bacteria, an oxidation reaction occurs and a large number of free radicals will be produced (Li *et al*., 2021). ·OH, O_2-· and H_2O_2 are the three most representative free radicals, and they are significantly related to the aging of organisms and the occurrence of many diseases (Granger and Kvietys,2015). O_2-· not only has its toxicity, but also can generate other reactive oxygen radicals through a series of reactions, which attack the polyunsaturated fatty acids in biofilms and trigger lipid peroxidation, thus forming lipid peroxides (ROOH), such as MDA, ketone group, hydroxyl group, etc., further causing damage to organisms. The level of MDA can often reflect the degree of lipid peroxidation and the severity of free radical attack on the body cells (Ma *et al*., 2021). XOD is a nucleotide catabolic enzyme, which can catalyze the oxidation of hypoxanthine to xanthine and then to uric acid. It can utilize molecular oxygen as an electron acceptor to generate a large amount of reactive oxygen species (ROS) through a cascade reaction, including O_2-•, •OH, H_2O_2, etc. (Zhang *et al.*, 2021). The body's regular metabolism will produce free radicals, and the body can also effectively eliminate free radicals. The elimination of long-lived free radicals such as O_2-·, H_2O_2 and ROOH is mainly achieved by the enhancement of the activities of SOD, CAT and other enzymatic systems to complete the antioxidant effect of the cell, so the activities of CAT and SOD can reflect the body's antioxidant function (Granger *et al*.,2015). The study showed that *Suaeda rigida* extract could significantly promote the activities of SOD, CAT in the serum of Karakul sheep, and inhibit the contents of MDA, H_2O_2, •OH and the XOD activity to varying degrees. With the increase of addition time, the inhibition of MDA, H_2O_2, •OH and the promotion of SOD and CAT became more and more apparent. The results indicated that the antioxidant capacity of Karakul sheep serum was significantly increased by adding an appropriate amount of *Suaeda rigida* extract to the diet, and its mechanism was related to scavenging free radicals in the body and enhancing the activity of antioxidant enzymes in vivo.

Effects of *Suaeda rigida* extract supplementation on immune function of Karakul sheep

Serum total protein is mainly composed of globulin and albumin, which generally reflect the antibody content, resistance level, body nutritional status and protein metabolism level of animals. Generally speaking, the body's nutritional status is good, the protein synthesis is increased, and the content of serum total protein is increased, which indicates that the animal's immunity level is increased. Globulins are secreted by hepatocytes and immune cells, and are the main component of the body's humoral immunity. Its content reflects the body's resistance to disease (Lv *et al*., 2021). IgG, IgA and IgM are the three most essential and abundant immunoglobulins in the body. Among them, IgG and IgA have the immune effects of antibacterial, antiviral, neutralizing toxin, enhancing the functions of phagocytes and killer cells (Li *et al*., 2021). IgM is the main antibody of the primary immune response, which can activate the classical pathway of complement as well as IgG. In addition, the contents of total protein, albumin and globulin in serum were mainly affected by the contents of protein in feed (Shedeed *et al*., 2019). The results showed that the contents of total protein, globulin, IgG, IgA and IgM in the serum of Karakul Sheep of the experimental group were significantly higher than those of the control group, and the white globulin ratio was lower than that of the control group, indicating that the protein content in *Suaeda rigida* extract was higher. The addition of *Suaeda rigida* extract to the diet could increase the protein synthesis, disease resistance, immunity and nutritional status of Karakul Sheep. These results were in agreement with those reported by Zhang *et al.* (2021), they found that the contents of three immunoglobulins were improved by supplementation of the rosemary extract to dairy goats. These results might be attributed to the biologically active compounds such as polysaccharides, alkaloids and flavonoids, which positively affect the humoral immune response (Shedeed *et al*., 2019).

In conclusion, the growth performance, anti-oxidation, anti-aging ability and immune function of Karakul sheep were significantly improved by adding 20 g/kg of *Suaeda rigida* extract in the basal diet. However, the exact components of *Suaeda rigida* extract that play these roles need to be further studied. The study proved that *Suaeda rigida* extract has good

feeding value and is a Chinese herbal feed additive and forage with regional advantages. Feeding Karakul sheep with its proper amount can not only reduce the feeding cost, but also improve the green degree of herding products, which has guiding significance for expanding the resources of *Suaeda rigida*.

CONFLICT OF INTEREST

The authors have declared that there were no conflicts of interest.

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