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Effect of Dietary Supplementation of Astragaloside IV on Growth performance, Inflammatory, and Antioxidant Status of Holstein Male Calves

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ABSTRACT

Holstein male calves were selected to study the effects of supplementing diets with Astragaloside IV (ASIV) on growth performance, inflammatory, and antioxidant functions. The calves were assigned to four treatment groups with six calves each in a completely randomized design. The calves were fed diets in which different amounts of ASIV were provided (0, 15, 30, or 60 mg/d per calf). The experimental period consisted of 7 days of adaptation followed by 120 days of data collection. Calves were fed 6 L/day of milk replacer from 7–60 days, weaned at 60 days, and offered water, starter, and Chinese wildrye *ad libitum* for the whole trial period. The total dry matter intake of calves were similar among treatments, whereas the final body weight at 120 days (P = 0.084) and average daily gain (P = 0.025) increased with increased ASIV. There were no significant differences among treatments on body measurement indexes. Serum blood urea nitrogen levels decreased (P = 0.028) and glucose levels increased (P = 0.029) with increasing ASIV. The average concentrations of CAT (P = 0.0005), GSH-Px (P < 0.0001), and T-SOD (P = 0.013) increased, and MDA (P = 0.006) decreased with increased feeding amount of ASIV. Furthermore, concentrations of IL-6, IL-8, and TNF-α were not affected by treatments (P >0.05). In conclusion, ASIV improved the growth performance and antioxidant function of calves in a concentration-dependent manner. To further study the mechanisms underlying the action of ASIV to improve antioxidant functionality and immune level *in vitro* experiments should be explored.

INTRODUCTION

The survival rate of calves is one of the main factors limiting the development of the cattle industry, and healthier calves are required to increase productivity. When the rumen and digestive tract of calves are not fully developed, they are susceptible to external environmental conditions, feeding methods, and a range of different factors, which can lead to oxidative stress. Consequently, animals may suffer from indigestion, impaired immune function, slow growth, and development, or even death (Terré *et al*., 2007). At present, calf health can be evaluated by determining body measurement changes and serum metabolic indicators, especially their antioxidant capacity and immune level.

The traditional method consists of administering antibiotics to livestock, and can reduce morbidity and mortality (Wileman *et al*., 2009). However, the adaptation of bacteria to become resistant to these antibiotics hinders the development of animal husbandry and has important consequences on human health (Thames *et al*., 2012). The removal of antimicrobials from poultry, swine, cattle and other livestock diets has triggered a search for suitable natural alternatives (Acamovic and Cross,

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2007). Therefore, it is important to find safe and effective antioxidant drugs to slow down the occurrence of disease in calves.

Chinese herbal medicines or plant extracts are characterized by low toxicity and are affective. *Astragalus membranaceus,* a commonly used Chinese medicinal plant, has been shown to have pharmacological benefits (Qi *et al*., 2014; Li *et al*., 2017a). Astragaloside IV (ASIV) is the main active ingredient of *Astragalus* saponins, and its anti-inflammatory, anti-oxidative, and immune-regulatory activities have been previously demonstrated (Ren *et al*., 2013; Li *et al*., 2017b). Many previous studies have shown that ASIV plays an important role in the clinical treatment of atherosclerosis, cardiac fibrosis, diabetes, liver damage, and other diseases (Dai *et al*., 2017; He *et al*., 2017; Li *et al*., 2018; Sun *et al*., 2018). However, there have been few studies that have investigated the effects of supplemented ASIV on animals, especially calves.Therefore, our study investigated the effects of ASIV supplementation on the performance, plasma biochemistry, antioxidant functions, and immune indexes of calves.

MATERIALS AND METHODS

Astragaloside IV

The astragaloside IV (ASIV) extract used in this study was purchased from a commercial pharmacy with a purity of 500 g/kg dry matter (Nanjing Spring and Autumn Biological Engineering Co. Ltd, Nanjing, China). The extract was prepared by extracting from the roots of the leguminous plant *Astragalus* and purified with high performance liquid chromatography.

Animals, diets, and experimental design

The experiment was conducted under experimental license from the Institutional Animal Care and Use Committee (IACUC20060101, 1 Jan 2006) of the Shandong Academy of Agricultural Sciences.

The experiment was conducted at the Shandong Qingye Pasture Co., Ltd from July 2018 to November 2018. We selected 24 Holstein male calves (7 days after birth) with an initial body weight of 40.79 ± 2.77 kg (mean ± SD). They were housed in individual pens and provided free access to water by bucket. Calves were randomly allocated to four groups of six calves (n = 6).

Four treatment groups received different doses of ASIV mixed into the diet (0, 15, 30, and 60 mg/d per calf). The ASIV powder was mixed with 50 ml water, and feeding was done by mixing it in milk replacer liquid or through oral syringe. The experiments consisted of two periods (i) 0–60 days when calves were fed milk replacer (Beijing Precision Animal Nutrition Research Center, Beijing, China), the milk replacer was prepared fresh in 1:7 power to water (lukewarm) ratio and fed twice per day (at 8:00 and 16:00) for a total of 6 L per cattle, and Chinese wildrye and starter (Shandong Jiurui Agricultural Group Co., Ltd, Shandong, China) *ad libitum*. After 60 days, calves were weaned, (ii) and 60–120 days when calves were only fed Chinese wildrye and starter *ad libitum*. The chemical composition of milk replacer powder, Chinese wildrye, and starter are listed in Table I.

Table I. The chemical composition of the milk replacer powder, Chinese wildrye, and starter.

Items	Milk replacer	Chinese wildrye	Starter
Dry matter, % of fresh basis	94.68	92.28	89.28
Nutrient composition†			
Crude protein	22.93	6.61	22.25
Ether extract	16.02	1.74	5.64
Neutral detergent fiber	5.07	69.79	24.85
Acid detergent fiber	1.52	41.76	7.29
Ash	4.30	5.34	8.59
Calcium	0.90	0.27	1.00
Phosphorus	0.49	0.36	0.45

† % of DM.

Growth performance traits

Throughout the experiment, the amount of diet offered and refused was recorded daily to calculate dry matter intake (DMI). Body weight was continuously measured before the morning feeding in the last 3 days of each period. Average daily gain (ADG) of the calves were calculated between feeding period intervals. The feed conversion rate (FCR) was calculated by the ratio of DMI: ADG.

Body measurement indices

Body measurement indexes were measured and recorded after body weighing, and included whither height (WH), body length (BL), and heart girth (HG); they were measured in an unforced position. To study the relative growth of calves, the body length index (BLI), heart girth index (HGI), and somatic index (SI) were calculated with the following equations (Mavule *et al*., 2013; Liu *et al*., 2018): BLI=BL/WH, HGI=HG/WH, and SI=HG/BL.

Serum sampling and analysis

Approximately 10 ml of blood was collected from the jugular vein prior to the morning feeding on the last day of each period. Blood was collected in 10-ml centrifuge

tubes and directly centrifuged at 3000 rpm for 30 min at room temperature. Separation of serum was subdivided into three portions and frozen at −20°C for determining biochemical, antioxidant, and immune indicators. Serum biochemical indicators, including high density lipoprotein (HDL), low density lipoprotein (LDL), glucose (GLU), total protein (TP), blood urea nitrogen (BUN), triglyceride (TG), total cholesterol (T-CH), alanine aminotransferase (ALT), and aspartate transferase (AST) were measured using an automatic biochemical analyzer (7100, Hitachi, Tokyo, Japan). Serum antioxidant indicators, including total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), catalase (CAT), total antioxidant capacity (TAC), and malondialdehyde (MAD) were determined using commercially available assay kits (Nanjing Jiancheng Institute of Bioengineering Institute China). Serum immune indicators, including interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) were detected using enzyme-linked immunosorbent assay kits (Jinan Jianbang Biotechnology Co., Ltd., Shandong, China).

Statistical analysis

All data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, 2002) based on the following statistical model:

$$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + A_k + e_{ijk,}$$

where μ = overall mean; T_i = fixed effect of ASIV treatments (i = 1 to 4); P_j = fixed effect of the period within two feeding periods (j = 1 to 2); $(T \times P)_{ij}$ = fixed effect of interaction between treatment and period; A_k = random effect of animals (k = 1 to 6), and e_{ijk} represents the random residual error. P <0.05 indicated a significant difference. A tendency towards a difference was also considered for 0.05 ≤ P <0.10. Polynomial analysis was conducted to determine the quadratic or linear response to the increasing ASIV dosage in the diet. The means of each trait were compared by Turkey multiple comparisons if a significant treatment effect was found.

RESULTS

Growth performance

The results of growth performance are shown in Table II. The BW of calves at 60 days was not affected by the different diets (P >0.05), whereas the BW at 120 days linearly increased with increased ASIV (P= 0.084). The DMI was not affected by the different diets during the two periods and on average (P >0.05). Except for during the first 60 days, the ADG linearly increased with increased ASIV during the 60–120-day period and on average (P <0.05). The FCR was no affected during the two periods, but significance declined on average (P =0.014).

Table II. Effect of Astragaloside IV on growth performance of Holstein calves.

Items	Feeding amount of ASIV (mg/d)				SEM	P-values				
	0	15	30	60		T	P	T × P	L	Q
Initial BW, kg	43.00	37.25	43.00	39.90	1.13	0.248				
Final BW, kg										
60 d	78.15	74.55	80.15	77.20	1.07	0.416	<0.001	0.193	0.766	0.883
120 d	126.02	130.75	136.78	134.06	1.65	0.084			0.028	0.288
TDMI[1], g/d										
0–60 d	1147.93	1148.60	1152.10	1153.90	1.07	0.124	<0.001	0.159	0.021	0.778
60–120 d	2399.00	2404.50	2472.00	2422.80	11.95	0.145			0.158	0.239
Mean	1773.47	1776.55	1812.05	1788.35	6.17	0.125			0.114	0.258
ADG, kg/d										
0–60 d	0.58	0.62	0.62	0.62	0.01	0.471	<0.001	0.370	0.236	0.425
60–120 d	0.82	0.94	0.94	0.95	0.02	0.082			0.037	0.188
Mean	0.70b	0.78a	0.77a	0.79a	0.03	0.025			0.003	0.041
FCR										
0–60 d	1.97	1.85	1.87	1.87	0.03	0.447	<0.001	0.401	0.269	0.347
60–120 d	2.96	2.57	2.64	2.56	0.07	0.099			0.054	0.243
Mean	2.53a	2.28b	2.32b	2.28b	0.04	0.014			0.011	0.096

a, b, c Means within a row with different subscripts differ when $P_{treatment}$ < 0.05. [1]Total Dry matter intake included Milk replacer, Chinese wildrye and Starter dry matter intake. BW, body weight; TDMI, total dry matter intake; ADG, average daily gain; FCR: feed conversation rate; T, treatment of fixed effect; P, period of fixed effect; T × P, the interaction within treatment and period of fixed effect; L, linear response to the Astragaloside IV treatment; Q, quadratic response to the Astragaloside IV treatment.

Table III. Effect of Astragaloside IV on the body measurement indices of Holstein calves.

Items	Feeding amount of ASIV (mg/d)				SEM	P-values				
	0	15	30	60		T	P	T × P	L	Q
BLI										
0–60 days	1.40	1.55	1.59	1.35	0.071	0.632	0.882	0.418	0.840	0.211
60–120 days	1.13	1.51	1.61	1.57	0.089	0.151			0.061	0.224
Mean	1.27	1.53	1.60	1.46	0.056	0.165			0.217	0.109
HGI										
0–60 days	1.46	1.64	1.96	1.37	0.082	0.051	0.001	0.684	0.921	0.015
60–120 days	2.08	1.94	2.41	2.18	0.127	0.703			0.510	0.867
Mean	1.78	1.79	2.19	1.77	0.088	0.194			0.534	0.173
SI										
0–60 days	1.04	1.07	1.29	1.03	0.049	0.241	0.001	0.341	0.650	0.139
60–120 days	1.88	1.31	1.70	1.41	1.121	0.315			0.327	0.563
Mean	1.46	1.19	1.50	1.22	0.076	0.238			0.485	0.989

a, b, c Means within a row with different subscripts differ when $P_{treatment} < 0.05$. BLI, body length index; HGI, heart girth index; SI, somatic index.

Body measurement indices

The results of body measurement indexes of calves are shown in Table III. The amount of ASIV had no effect on the BLI of calves from 0–60 day ($P > 0.05$) and on average, and had tendency effects from 60–120 days ($P = 0.061$). The HGI of calves fed 30 mg/d of ASIV was higher than that of other diets ($P = 0.051$). However, there was no difference among treatments from 60–120 days and on average ($P > 0.05$). The SI was not affected by different diets during the two periods and on average ($P > 0.05$).

Serum analysis

The results of serum biochemical index of calves are shown in Table IV. The HDL, LDL, ALT, AST, and TCH content of calves were not affected by different diets during the two periods and on average ($P > 0.05$). The TP content linearly increased and BUN content linearly decreased with increased ASIV from 0–60 days ($P < 0.05$). GLU content linearly increased from 0–120 days ($P < 0.05$). Moreover, the TP content from 0–60 days and GLU content on average were higher, and BUN and TG content from 0–60 days was lower for calves fed 30 mg/d of ASIV than that of the other diets ($P < 0.05$).

The results of serum antioxidant index of calves are shown in Table V. From 0–60 days, CAT and GSH-Px content linearly increased ($P < 0.05$), and T-SOD content tended to linearly increase ($P = 0.098$), and MDA content tended to linearly decrease ($P = 0.076$) with increased ASIV. From 60–120 days, CAT and T-SOD content linearly increased ($P < 0.05$), and MDA content linearly decreased ($P = 0.035$) with increased ASIV. On average, the serum antioxidant index of calves had similar varying tendencies to those from 0–60 days. Moreover, the TCA content was not affected by the different diets during the two periods or on average ($P > 0.05$).

The results of serum immune index of calves are shown in Table VI. The IL-6, IL-8, and TNF-α content were not affected by the different diets during the two periods and on average ($P > 0.05$).

DISCUSSION

In the present study, supplemented ASIV had no effect on DMI, whereas the result showed a significant increase in the final body weight and ADG on average. Although some studies have reported that *Astragalus polysaccharides* could improve ADG and tended to increase the food intake of weaned pigs; however, the FCR did not improve (Yuan *et al*., 2006). Ying (2010) also observed that basal diet supplementation with *Astragalus* polysaccharides could increase body weight improving broiler growth performance. However, there are few studies on ASIV in ruminants at present, and body weight is an important indicator for evaluating growth and development (Heinrichs *et al*., 1992). In our results, the increase of calves weight is closely related to the addition of ASIV into the diet to improve the intestinal environment of calves and promote the absorption of nutrients.

The body measurement indexes can reflect the relative body shape regardless of growth and nutritional status of calves. We found that ASIV supplementation did not affect body length parameters, including BLI, HGI, and SI. This result indicated that the body size ratio was appropriate and well developed.

Table IV. Effect of Astragaloside IV on serum biochemical index of Holstein calves.

Items	Feeding amount of ASIV (mg/d)				SEM	P-values				
	0	15	30	60		T	P	T × P	L	Q
HDL (mmol/L)										
60 days	1.44	1.67	1.50	1.47	0.13	0.952	0.138	0.998	0.936	0.669
120 days	1.11	1.42	1.17	1.20	0.12	0.849			0.988	0.600
Mean	1.28	1.54	1.33	1.33	0.09	0794			0.964	0.557
LDL (mmol/L)										
60 days	3.44	2.77	2.81	3.26	0.14	0.262	<0.001	0.912	0.678	0.063
120 days	2.02	1.68	1.64	1.89	0.12	0.622			0.674	0.231
Mean	2.73	2.22	2.22	2.58	0.14	0.132			0.578	0.043
ALT (U/L)										
60 days	6.57	6.82	6.19	6.35	0.30	0.930	<0.001	0.293	0.649	0.949
120 days	18.55	17.50	19.81	20.61	0.65	0.366			0.162	0.481
Mean	11.89	12.16	13.97	12.69	1.17	0.581			0.383	0.331
AST (U/L)										
60 days	40.88	42.61	45.21	42.43	2.07	0.917	<0.001	0.841	0.718	0.628
120 days	80.65	87.00	80.09	85.94	3.31	0.870			0.791	0.973
Mean	54.14	61.64	62.65	64.18	4.07	0.902			0.385	0.277
TP (g/L)										
60 days	42.93b	42.03b	47.81a	47.23a	0.77	0.002	0.292	0.717	0.001	0.870
120 days	40.05	45.72	56.74	55.88	4.21	0.443			0.140	0.707
Mean	41.49	43.87	52.27	51.55	2.14	0.199			0.292	0.086
BUN (mmol/L)										
60 days	3.95a	3.69ab	3.11b	3.42ab	0.12	0.038	<0.001	0.814	0.018	0.160
120 days	4.59	4.62	3.90	4.47	0.15	0.430			0.503	0.238
Mean	4.20a	4.00a	3.45b	3.89ab	0.11	0.028			0.410	0.331
TCH (mmol/L)										
60 days	1.82	1.42	1.61	1.78	0.08	0.334	0.568	0.380	0.894	0.117
120 days	1.63	1.94	1.79	1.64	0.13	0.834			0.914	0.410
Mean	1.73	1.68	1.72	1.71	0.07	0.998			0.410	0.339
TG (mmol/L)										
60 days	0.29a	0.22ab	0.10b	0.19ab	0.03	0.038	0.018	0.048	0.206	0.363
120 days	0.25	0.27	0.30	0.31	0.02	0.709			0.268	0.904
Mean	0.27	0.24	0.20	0.25	0.012	0.490			0.378	0.269
GIu (mmol/L)										
60 days	5.29	7.35	6.64	6.41	0.38	0.279	0.069	0.334	0.387	0.170
120 days	5.05b	5.46b	6.58a	5.84ab	0.18	0.005			0.005	0.041
Mean	5.16b	6.27b	6.60a	6.13ab	0.20	0.029			0.410	0.331

a, b, c Means within a row with different subscripts differ when $P_{treatment} < 0.05$. HDL, high density lipoprotein; LDL, low density lipoprotein; Glu, glucose; TP, total protein; BUN, blood urea nitrogen; TG, triglyceride; TCH, total cholesterol; ALT, alanine aminotransferase; AST, aspartate transferase.

Serum biochemical indexes can be indicators for the status of metabolic and physiological functions in animals. Thus, they can reflect the antioxidant and immune functions of calves. TP has been regarded as a

Table V. Effect of Astragaloside IV on serum antioxidant index of Holstein calves.

Items	Feeding amount of ASIV (mg/d)				SEM	P-values				
	0	15	30	60		T	P	T × P	L	Q
CAT (U/ml)										
60 days	0.51	0.94	1.39	1.24	0.14	0.078	0.767	0.964	0.023	0.280
120 days	0.41b	0.79ab	1.39a	1.31a	0.13	0.007			0.001	0.261
Mean	0.46c	0.85bc	1.39a	1.27ab	0.10	0.0005			0.0003	0.222
GSH-Px (U/ml)										
60 days	9830b	10380b	13380a	11640ab	421.66	0.006	0.660	0.146	0.006	0.086
120 days	10820b	11400b	13140a	10548b	301.55	0.005			0.623	0.003
Mean	10325b	10890b	13260a	11094b	256.14	<.0001			0.004	0.001
T-AOC (U/ml)										
60 days	2.26	2.81	2.31	2.27	0.20	0.780	0.506	0.516	0.502	0.076
120 days	2.38	2.84	2.44	2.84	0.15	0.606			0.485	0.937
Mean	2.15	2.82	2.64	2.55	0.12	0.248			0.291	0.113
T-SOD (U/ml)										
60 days	108.92	130.70	135.69	131.70	5.18	0.202	<0.001	0.536	0.098	0.213
120 days	146.27b	147.17b	178.85a	166.22ab	4.77	0.030			0.015	0.399
Mean	125.90b	138.94ab	157.27a	148.96a	4.49	0.013			0.005	0.088
MDA (nmol/ml)										
60 days	1.81	1.37	1.19	1.42	0.09	0.086	0.232	0.826	0.076	0.067
120 days	1.62	0.98	0.94	0.90	0.13	0.080			0.035	0.197
Mean	1.76a	1.21b	1.07b	1.17b	0.08	0.006			0.008	0.035

a, b, c Means within a row with different subscripts differ when $P_{treatment} < 0.05$. CAT, catalase; GSH-Px, glutathione peroxidase; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase; MDA, malondialdehyde.

Table VI. Effect of Astragaloside IV on serum immune index of Holstein calves.

Items	Feeding amount of ASIV (mg/d)				SEM	P-values				
	0	15	30	60		T	P	T × P	A	B
IL-6 (pg/ml)										
60 days	132.40	125.55	124.02	121.66	7.34	0.960	0.980	0.994	0.625	0.892
120 days	130.31	124.39	123.07	125.99	6.74	0.985			0.860	0.747
Mean	131.36	124.97	123.55	123.82	4.92	0.945			0.639	0.754
IL-8 (pg/ml)										
60 days	302.43	260.82	289.74	277.35	12.56	0.715	0.521	0.997	0.685	0.592
120 days	291.32	24.83	276.90	271.85	10.91	0.544			0.935	0.873
Mean	296.88	252.82	283.32	274.60	8.25	0.349			0.698	0.432
TNF-α (ng/ml)										
60 days	1.32	1.29	1.22	1.28	0.08	0.977	0.019	0.818	0.772	0.811
120 days	1.75	1.70	1.72	1.41	0.11	0.709			0.343	0.590
Mean	1.53	1.50	1.47	1.34	0.07	0.787			0.207	0.682

a, b, c Means within a row with different subscripts differ when $P_{treatment} < 0.05$. IL-6, interleukin-6; IL-8, interleukin-8; TNF-α, tumor necrosis factor-α.

carrier of nutrients and content changes reflect dietary protein levels. Therefore, decreased TP content indicates that the nutrient content of the protein was insufficient (Bartlett *et al*., 2006). In this experiment, the TP content was significantly higher than that of the control group at 60 days, and it also showed a tendency to increase at 120 days. This result indicated that ASIV may promote protein absorption, reduce protein decomposition, and consequently affect calf body weight, which is consistent with the results pertaining to growth performance. In ruminants, BUN is mainly metabolized by the liver, part of which is excreted from the body, and part of which is returned to the intestine for further anabolism for use in the body (Lapierre and Lobley, 2001). Therefore, BUN is an important indicator reflecting nitrogen metabolism, amino acid balance, and energy nitrogen balance (Abe *et al*., 1997). The levels of BUN were significantly decreased during the feeding trails. Thus, it was beneficial to amino acid balance and nitrogen utilization and improved the utilization efficiency of protein in the body. This also increased the body weight of calves. Both TG and TCH are neutral fats, which are important indicators of blood lipid levels and reflect the body's lipid metabolism. HDL and LDL are two lipoproteins in serum. LDL can transport TCH, while HDL is an anti-atherosclerotic lipoprotein that can transport cholesterol in surrounding tissues. In the present study, there was no significant effect of the ASIV treatments on TG, TCH, HDL, or LDL. This result indicated that ASIV did not cause abnormal lipid metabolism in calves and had no adverse effects on the health of the body. GLU is a biochemical indicator of energy metabolism, which can reflect energy levels (Stanley *et al*., 2002). Our results suggested that GLU was not affected up to 60 days but increased from 60–120 days, which might have been induced by rumen and digestive tract development. Furthermore, ASIV supplementation promoted digestion and metabolism of carbohydrates, which was conducive to maintain energy balance. AST and ALT are important indicators reflecting heart and liver function, respectively. When liver tissue was damaged, serum transaminase activity would increase (Wang *et al*., 2015). The ASIV had no influence on AST and ALT, indicating that ASIV supplementation did not affect the heart and liver of calves.

In healthy animals, the production and elimination of free radicals are balanced. When they become unbalanced, substances in the cells are excessively oxidized, which leads to oxidative stress (Sohal and Allen, 1990). GSH-Px can eliminate lipid peroxidation products. T-SOD and CAT are the main antioxidant enzymes in organisms and have strong abilities for scavenging free radicals. TCA is an important part of the antioxidant system, and can measure the total antioxidant capacity of the body. MDA is the end product of lipid peroxidation, which can reflect the oxidative stress state. Our results indicated that ASIV had a significant increases in the GSH-Px, T-SOD, CAT levels, and marked decreases in MDA. Although the effect of TCA was not significant, it generally increased. Our results were consistent with prvious reports. This might account of ASIV prevents animal damage by increasing the levels of antioxidant enzymes.

Other studies have also demonstrated the improvement of antioxidant status by supplementation with *Astragalus* polysaccharides in the diet of rats, lambs, and broilers (Yan *et al*., 2010; Zhong *et al*., 2012; Shengjun, 2018). The enhanced antioxidant enzyme activity and antioxidant status is perhaps due to bioactive compounds in ASIV, and those compounds possess different biological and pharmaceutical activities, such as antioxidant and free radical scavenging functions (Hao *et al*., 2018; Liu *et al*., 2017; Wang and Guo, 2019). Therefore, our results showed that ASIV could protect against oxidative stress by enhancing the body's antioxidant capacity.

Cytokines are produced by a variety of cells and have polypeptide molecules that regulate cell functions. They can participate in immune response and regulation and contribute to the prevention, diagnosis, and treatment of diseases. They are also typical inflammatory mediators of IL-6, secreted by T cells, macrophages, and smooth muscle cells. IL-6 stimulates inflammation and autoimmune processes against a range of diseases, such as atherosclerosis and diabetes. It can act as a pro-inflammatory cytokine and an anti-inflammatory cytokine by inhibiting IL-1 and TNF-α (Chen *et al*., 2019). IL-8 attracts and activates neutrophils, which are in contact with neutrophils and undergo morphological changes and release active substances, leading to local inflammatory reactions. TNF-α is secreted by macrophages and participates in the body's immune response as a pro-inflammatory cytokine (Pedersen and Bruunsgaard, 2003). The result of this experiment showed that ASIV had no significant effect on IL-6, IL-8, or TNF-α, indicating that ASIV supplementation did not cause an inflammatory response in calves. Meanwhile, ASIV can protect the health of the animal by inhibiting the damage of adverse external factors.

CONCLUSIONS

Based on the obtained results, ASIV supplementation in calves inhibited inflammatory response, and evidently improved the growth performance, increased the activity of serum antioxidant enzymes, and decreased the MDA content. To sum up, ASIV can enhance the

inflammatory and antioxidant capacity of calves. Based on the requirement for improved growth performance, it is recommended to feed Holstein male calves 30 mg/day.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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