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Astragalus polysaccharide (APS) supplement in beagle dogs after castration: Effects on the haematology and serum chemistry profiles, immune response, and oxidative stress status

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Abstract

Background: Castration is one of the most common surgical procedures performed in dogs. However, based on increasing evidence, male animals experience significant pain after castration. Astragalus polysaccharide (APS), one of the main bioactive components in A. membranaceus bunge, has been widely used as part of Fu-Zheng therapy to enhance natural defense mechanisms.

Introduction: This study was carried out to determine the effects of supplementing different doses of Astragalus polysaccharide (APS; control, 0 mg/kg; APSL, 400 mg/kg; and APSH, 800 mg/kg) for 8 weeks on the haematology and serum chemistry profiles, immune response, and oxidative stress status in weanling beagle dogs.

Methods: After adapting to the experimental environment for 1 week, 18 male beagle dogs (Sichuan Institute of Musk Deer Breeding, China; average initial weight, 3.80 ± 0.43 g; age, 3-month-old) were randomly allotted to diets supplemented with three doses of APS (Control, 0 mg/kg; low, 400 mg/kg; and high, 800 mg/kg), referred to as control, APSL, and APSH, respectively; six dogs were assigned to each treatment. The dogs were fed the respective diets twice daily at 08:30 and 16:30 h in sufficient quantity to supply the metabolizable energy requirements for 8 weeks. On day 43 (19 weeks old), the dogs were castrated. On days 42 (prior to castration, 19 weeks old), 50 (day 7 after castration, 20 weeks old), and 57 (day 14 after castration, 21 weeks old) to measure the haematology, blood chemistry, immune response, and oxidative stress status parameters.

Results: Based on our findings, the APSH diet decreased weight gain and increased the feed to gain ratio in dogs ($P < 0.05$). At 14 days after castration, the wound was almost closed, slightly swollen, dry, and clean in the groups supplemented with APS. In addition, optimal APS supplementation was found to decrease erythrocyte count

(RBC), haematocrit (HCT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), C-reactive protein (CRP), interleukin 1β (IL-1β), and tumor necrosis factor-α (TNF-α) levels, and cortisol and protein carbonyl (PC) concentrations ($P < 0.05$). Moreover, the mean corpuscular haemoglobin (MCH) and platelet (PLT) levels, interleukin 10 (IL-10) and glutathione (GSH) content, and Cu/Zn superoxide dismutase (SOD1), catalase (CAT), and glutathione peroxidase (Se-GPx) activities were increased in the APS supplemented groups ($P < 0.05$)

Conclusion: This study demonstrated that supplementing weanling beagle dogs with optimum APS could positively affect wound healing by improving their haematological profile (decreased RBC and HCT content, increased MCH and PLT levels), serum biochemical parameters (decreased ALP and ALT content), immune status (decreased CRP, IL-1β, and TNF-α levels; increased IL-10 content), and antioxidant defense (decreased cortisol and PC content; increased GSH content, and SOD1, CAT, and Se-GPx activities). However, the detailed mechanism whereby APS regulates these changes requires further investigation. In addition, the results of this study suggest that 400 mg/kg diet is the optimum APS dose for beagle dogs.

KEYWORDS
antioxidant defense, Astragalus polysaccharides, haematology and serum chemistry profile, immune response, weanling beagle dogs

1 | INTRODUCTION

Castration is one of the most common surgical procedures performed in dogs. In fact, castration has been shown to prevent diseases of the reproductive system, such as diseased testicles and enlarged prostates, and modify undesirable behaviors, including urine marking, wandering, undue sexual excitement, and intermale aggression (Aengwanich et al., 2019). However, based on increasing evidence, male animals experience significant pain after castration, which is disadvantageous in terms of physiological, behavioral, and health consequences, especially inflammatory and oxidative stress (Prunier et al., 2006). The percentage of neutrophils and the neutrophil/lymphocyte ratio were found to be higher after castration in dogs (Aengwanich et al., 2019). In calves, the scrotal inflammation score was found to be significantly higher than that in control animals (Olson et al., 2016). According to Bretschneider (2005), castration is an acute stressor, and plasma cortisol values are significantly elevated after the surgical procedure. Thus, animal welfare concerns are increasing pressure on farmers that perform castration (Prunier et al., 2006).

Astragalus polysaccharide (APS), one of the main bioactive components in A. membranaceus bunge, has been widely used as part of Fu-Zheng therapy to enhance natural defense mechanisms (Zhao et al., 1990). He et al. (2012) reported that APS inhibits the lipopolysaccharide-induced production of TNF-α and IL-1β. In addition, APS has been shown to enhance superoxide dismutase (SOD) activity and reduce reactive oxygen species (ROS) formation in mice (Chen et al., 2018). Accordingly, APS may minimize the negative consequences of castration in dogs by improving the immune response and oxidative stress status; however, studies have not been conducted to demonstrate this notion. The objective of this study was to determine the castrated beagle dogs were altered in the haematology and serum chemistry profiles, immune response, and oxidative stress status by supplementing different doses of APS, which could be used to preliminarily identify an APS-dependent mechanism that minimizes the pain and oxidative stress experienced by recently castrated animals.

2 | MATERIAL AND METHODS

2.1 | Animal experiments

After adapting to the experimental environment for 1 week (Queau et al., 2020), 18 male beagle dogs (Sichuan Institute of Musk Deer Breeding, China; average initial weight, 3.80 ± 0.43 g; age, 3-month-old) were randomly allotted to diets supplemented with three doses of APS (Control, 0 mg/kg; low, 400 mg/kg; and high, 800 mg/kg), referred to as control, APSL, and APSH, respectively; six dogs were assigned to each treatment. The experimental APS was the same as that used in our previous study. The nutrient requirements for the basal diet were based on the Association of American Feed Control Officials (AAFCO, 2010). The experimental cages ($1.4 \times 1.4 \times 1.4$ m^3) were housed in an air-conditioned room, where temperature and relative humidity were 21 ± 2°C and 55 ± 15%, respectively, and the photocycle was 14-h light/10-h dark, as described by Dayan et al. (1998). The dogs were

fed the respective diets twice daily at 08:30 and 16:30 h in sufficient quantity to supply the metabolizable energy requirements for 8 weeks (Sabchuk et al., 2019). The feed ration was adjusted every week with a slight increase in feed allowance as described by Dobenecker et al. (2013) and tap water ad libitum, as described by Ochi et al. (2013). On day 43 (19 weeks old), the dogs were castrated according to Hutchison (1976).

2.2 | Sample collection and handling

Dogs from each experimental cage were weighed at the initiation and every 2 weeks of the experiment. Twelve hours after the last feeding (Swanson et al., 2004), blood samples from each treatment group were withdrawn from the cephalic vein of the forelimb using an evacuated tube, according to Ochi et al. (2013), on days 42 (prior to castration,19 weeks old), 50 (day 7 after castration, 20 weeks old), and 57 (day 14 after castration, 21 weeks old) to measure the haematology, blood chemistry, immune response, and oxidative stress status parameters. A total of 10 ml of blood was collected from each treatment group at each collection time, and blood for haematology was collected using EDTA as an anticoagulant, while blood for serum chemistry analysis was collected without an anticoagulant. After clotting, serum was separated by centrifugation at 3000 × g for 8 min, stored at −80°C, and analyzed according to Alexander et al. (2018).

2.2.1 | Growth parameters

All beagle dogs were weighed at the start and at the end of the experiment to calculate final weight (FW) and weight gain (WG). The amount of daily intake was recorded to calculate the feed to gain (F:G) ratio. Values were calculated using the following formulae according to Kürekci et al. (2020).

$$\text{WG (kg)} = \text{FW} - \text{IW, where FW} = \text{final weight (kg)}$$
$$\text{IW (g)} = \text{initial weight (kg)}$$
$$\text{F : G} = \text{FI (kg)} / \text{WG (kg)}, \text{where FI} = \text{Food intake (kg)}.$$

2.2.2 | Hematology and blood chemistry parameters assays

Haematological parameters, such as leukocyte count (WBC), erythrocyte count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelet count (PLT), were estimated using an automated cell analyzer (KX-21, Sysmex Corporation, Kobe, Japan).

Biochemical parameters, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glutamyl transpeptidase (GGT), creatine kinase (CK), total protein (TP), albumin (ALB), globulin (GlO), albumin/globulin ratio (A/G), triglyceride (TG), and blood glucose (GlU), creatinine (CREA), blood urea nitrogen (BUN) and total bilirubin (TBIL) were estimated by spectrophotometry using a Mindray BS-420 Automatic Analyzer (Shenzhen, China).

2.2.3 | Immune response status and oxidative stress parameters assays

C-reactive protein (CRP) levels were measured by canine-specific ELISA, according to the manufacturer's instructions (Jiangsu Meibiao Biotechnology Co., Ltd., China, Cat No. MB-8813A). Serum levels of interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin 10 (IL-10), and transforming growth factor-β1 (TGF-β1) were analyzed with a canine-specific ELISA kit (Jiangsu Meibiao Biotechnology Co., Ltd., China); the minimum detectable doses for IL-1β, TNF-α, IL, 10, and TGF-β1 were typically less than 1.0, 0.1, 0.1, and 1.0 pg/mL, respectively.

The concentration of cortisol was measured by double antibody sandwich ELISA, as described by Daniel et al. (2008). The protein concentration of the samples was determined according to the method of Bradford (1976). Protein carbonyl (PC) content residue and malondialdehyde (MDA) were measured as described by Caperna et al. (2010) and Crnogaj et al. (2010), respectively. Cu/Zn superoxide (SOD1) and glutathione peroxidase (Se-GPx) activities were assayed as described by Zhang et al. (2008). Catalase (CAT) activity was assayed by the decomposition of hydrogen peroxide according to Aebi (1984). GSH content was determined according to the method described by Ayhanci et al. (2010). The intra-assay and inter-assay coefficient of variation for repeated measurement of CRP, IL-10, CAT, TGF-β1, IL-1β, TNF-α, Cortisol, PC, MDA, SOD1, GSH-Px, and GSH, content in samples were less than 6% and 10%, respectively.

2.3 | Statistical analysis

Results are expressed as mean ± standard deviation (SD). The growth performance data were analyzed using one-way analysis of variance (ANOVA). Repeated measures ANOVA were applied to compare the serum chemistry profiles, immune response, and oxidative stress status data during different times in each group. The effects of groups, time, and their interactions on the changes of each parameter were also analyzed. SPSS version 23 software (SPSS for Windows, SPSS, Inc.) was used for all statistical analyses. Statistically, significance level was considered to be $P < 0.05$.

3 | RESULTS

3.1 | The effect of APS on growth performance and wound healing

In this study, we assessed the growth performance of dogs. As shown in Table 1, FW and WG were the lowest for beagle dogs fed the diet

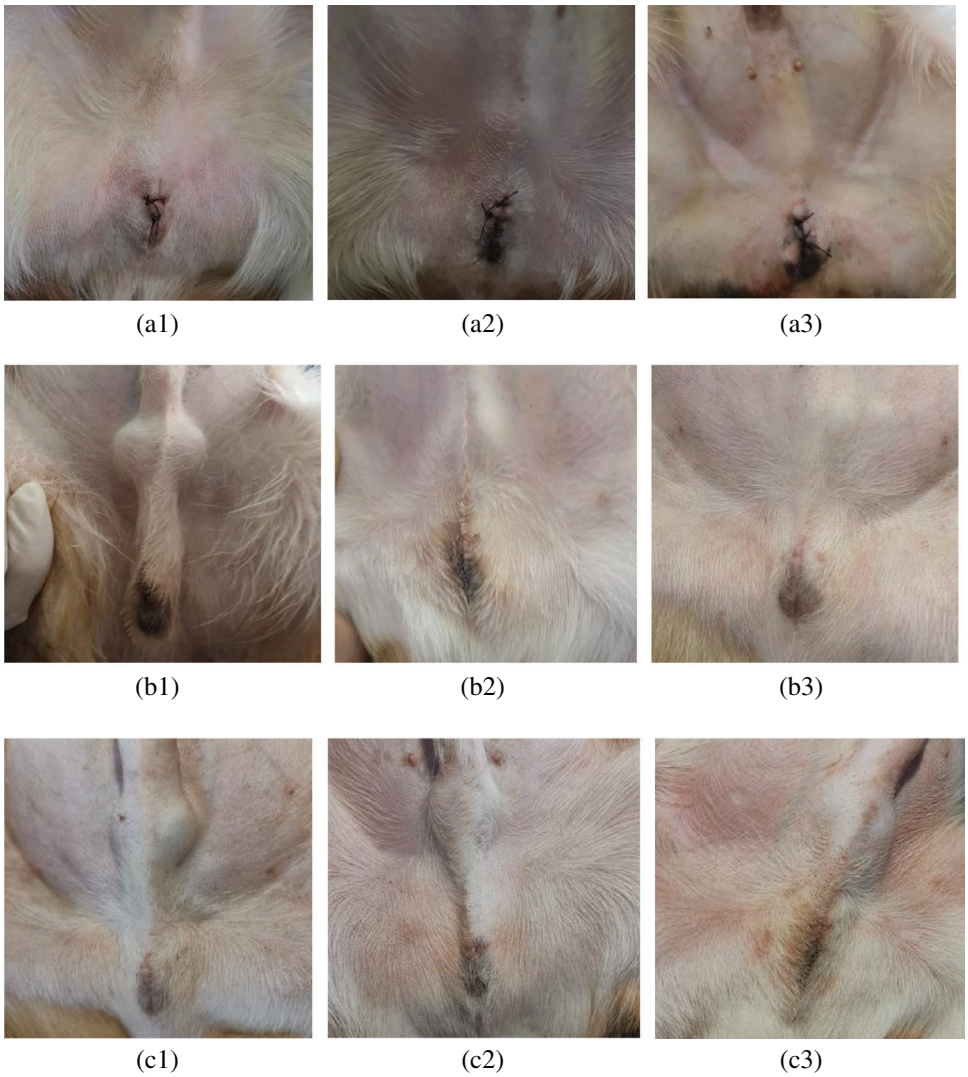

FIGURE 1 Effect of Astragalus polysaccharide (APS) supplementation on wound healing in beagle dogs. (Prior to castration: A1-Control, A2-APSL, A3-APSH; Day 7 after castration: B1-Control, B2-APSL, B3-APSH; Day 14 after castration: C1-Control, C2-APSL, C3-APSH)

TABLE 1 Growth parameters of beagle dogs supplemented with Astragalus polysaccharide (APS) for 56 days

Items	Control	APSL	APSH
IW	3.88 ± 0.60^{a}	3.85 ± 0.58^{a}	3.82 ± 0.13^{a}
FW	5.63 ± 0.35^{b}	6.00 ± 0.46^{b}	4.67 ± 0.23^{a}
WG	1.75 ± 0.26^{b}	2.15 ± 0.28^{b}	0.88 ± 0.10^{a}
F:G	7.98 ± 1.30^{a}	6.46 ± 0.81^{a}	15.69 ± 1.77^{b}

IW = initial weight; FW = final weight; WG = weight gain; F:G = feed to gain. Values are means ± SD (n = 6), and different superscripts in the same row are significantly different ($P < 0.05$).
Control: Astragalus polysaccharide-supplemented at dose 0 mg/kg diet.
APSL: Astragalus polysaccharide-supplemented at dose 400 mg/kg diet.
APSH: Astragalus polysaccharide-supplemented at dose 800 mg/kg diet.

supplemented with the high level of APS ($P < 0.05$); however, the F:G ratio was significantly higher in the APSH groups than in the APSL and control groups ($P < 0.05$). As described in Figure 1, the wound was almost closed, slightly swollen, dry, and clean in the APSL and APSH groups after surgical castration for 14 days, while in the control group, the wounds were poorly healed, open, wet with blood, and were draining small amounts of purulent material.

3.2 | Effect of APS on the haematology and blood chemistry parameters

The haematology parameters of beagle dogs supplemented with APS are shown in Table 2. The group × time interaction were significant in WBC, RBC, HCT, MCH, and PLT ($P < 0.05$). No significant differences were observed in WBC, HGB, MCV, and MCHC with increasing levels of APS at the same treatment time ($P > 0.05$). Additionally, there were no significant differences in RBC, HGB, HCT, MCV, and MCHC parameters between the same treatments at various time ($P > 0.05$). However, prior to castration, the levels of RBC and HCT in the APSL group were significantly lower than those in the control group ($P < 0.05$), while the

TABLE 2 Haematology parameters of beagle dogs supplemented with Astragalus polysaccharide (APS) for 56 days

		Time			*P* Value		
Items	Groups	Prior to castration	Day 7 after castration	Day 14 after castration	Group	Time	Group × Time
WBC (10^9/L)	Control	10.63 ± 1.53^{aA}	9.17 ± 0.95^{aA}	10.90 ± 0.75^{aA}	$P = 0.70$	$P < 0.01$	$P < 0.05$
	APSL	10.93 ± 1.02^{aA}	9.73 ± 1.16^{aA}	11.03 ± 1.16^{aA}			
	APSH	13.00 ± 1.13^{aB}	9.10 ± 1.68^{aA}	11.17 ± 1.62^{aAB}			
RBC (10^{12}/L)	Control	6.88 ± 0.27^{bA}	6.88 ± 0.09^{bA}	6.52 ± 0.16^{bA}	$P < 0.05$	$P = 0.30$	$P < 0.05$
	APSL	5.95 ± 0.36^{aA}	6.08 ± 0.23^{aA}	6.19 ± 0.17^{abA}			
	APSH	6.24 ± 0.39^{abA}	5.78 ± 0.45^{aA}	5.94 ± 0.19^{aA}			
HGB (g/L)	Control	147.33 ± 5.69^{aA}	145.33 ± 7.77^{aA}	145.67 ± 6.11^{aA}	$P = 0.20$	$P = 0.66$	$P = 0.06$
	APSL	134.67 ± 6.81^{aA}	139.33 ± 4.04^{aA}	142.33 ± 1.15^{aA}			
	APSH	141.33 ± 6.11^{aA}	136.33 ± 7.57^{aA}	137.33 ± 4.73^{aA}			
HCT(L/L)	Control	0.44 ± 0.02^{bA}	0.43 ± 0.02^{bA}	0.42 ± 0.02^{aA}	$P < 0.05$	$P = 0.10$	$P < 0.01$
	APSL	0.39 ± 0.02^{aA}	0.38 ± 0.02^{aA}	0.41 ± 0.01^{aA}			
	APSH	0.40 ± 0.02^{abA}	0.40 ± 0.01^{abA}	0.39 ± 0.01^{aA}			
MCV (fL)	Control	63.47 ± 0.99^{aA}	63.87 ± 0.96^{aA}	64.60 ± 0.95^{aA}	$P = 0.29$	$P < 0.01$	$P = 0.90$
	APSL	65.47 ± 1.10^{aA}	66.00 ± 1.54^{aA}	66.27 ± 1.72^{aA}			
	APSH	64.83 ± 1.46^{aA}	65.23 ± 1.70^{aA}	65.93 ± 2.05^{aA}			
MCH (pg)	Control	21.43 ± 0.21^{aA}	21.63 ± 0.61^{aAB}	22.33 ± 0.38^{aB}	$P < 0.05$	$P < 0.01$	$P < 0.05$
	APSL	22.43 ± 0.40^{bA}	23.63 ± 1.02^{bA}	23.00 ± 0.46^{aA}			
	APSH	22.67 ± 0.70^{bA}	22.93 ± 0.46^{abA}	23.10 ± 0.53^{aA}			
MCHC (g/L)	Control	337.67 ± 3.06^{aA}	339.00 ± 6.08^{aA}	345.33 ± 1.53^{aA}	$P = 0.14$	$P = 0.11$	$P = 0.25$
	APSL	345.67 ± 2.89^{aA}	347.33 ± 5.13^{aA}	347.00 ± 8.54^{aA}			
	APSH	349.67 ± 3.06^{aA}	341.67 ± 9.29^{aA}	350.67 ± 2.52^{aA}			
PLT (10^9/L)	Control	319.67 ± 18.15^{aB}	244.67 ± 37.85^{aA}	345.33 ± 39.11^{aB}	$P < 0.01$	$P < 0.01$	$P < 0.05$
	APSL	457.00 ± 21.79^{bB}	385.33 ± 46.50^{bA}	409.00 ± 26.66^{aAB}			
	APSH	377.33 ± 31.90^{bA}	328.00 ± 22.34^{bA}	342.33 ± 51.2^{aA}			

WBC = leukocyte count; RBC = erythrocyte count; HGB = hemoglobin; HCT = hematocri; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet. Values are means ± SD (n = 6). Different superscript lowercase letters indicate significant differences in same time among different treatment (Control, APSL, APSH) ($P < 0.05$). Different superscript uppercase letters indicate significant differences in same treatment (Control, APSL, APSH) among different treatment time ($P < 0.05$). Control, Astragalus polysaccharide-supplemented at dose 0 mg/kg diet. APSL, Astragalus polysaccharide-supplemented at dose 400 mg/kg diet. APSH, Astragalus polysaccharide-supplemented at dose 800 mg/kg diet.

levels of MCH and PLT were significantly higher in the APSL and APSH groups than in the control group ($P < 0.05$). On day 7 after castration, RBC and HCT levels were highest in the control group, and lowest in the APSH group ($P < 0.05$). The APSL group had significant increases in the levels of MCH and PLT compared with the control group ($P < 0.05$). On day 14 after castration, RBC levels in the control groups were higher than those in the APSL group ($P < 0.05$). The WBC was lower in APSH group than other treatments on day 7 after castration($P < 0.05$). At control group, the PLT was the lowest on day 7 after castration ($P < 0.05$).

The blood chemistry parameters in the serum of beagle dogs are presented in Table 3. The group × time interaction were not significant ($P > 0.05$). Prior to castration day, the APSL group had a significant decrease in serum ALT and ALP levels ($P < 0.05$). However, APS supplementation did not significantly change these levels in the other groups ($P > 0.05$). On day 7 after castration, APSL treatment also decreased the ALT and ALP content compared with control treatment ($P < 0.05$). On day 14 after castration, the ALP levels in the control group were higher than those in the APSL group ($P < 0.05$). Among the all treatment, GGT decreased significantly on day 7 after castration, and CREA and TG increased significantly on day 14 after castration ($P < 0.05$). In APSH group, the CK, BUN, and TBIL levels were the highest on day 7 after castration ($P < 0.05$). No significant difference was observed in the other groups ($P > 0.05$).

3.3 | The effect of APS on immune response

Table 4 presents the immune response in serum of beagle dogs supplemented with APS. There was a significant group × time interaction on

TABLE 3 Blood chemistry parameters of beagle dogs supplemented with Astragalus polysaccharide (APS) for 56 days

		Time			*P* Value		
Items	Groups	Prior to castration	Day 7 after castration	Day 14 after castration	Group	Time	Group × Time
AST (U/L)	Control	26.53 ± 3.78^{aA}	27.47 ± 4.58^{aA}	28.23 ± 1.59^{aA}	$P < 0.05$	$P = 0.84$	$P = 0.94$
	APSL	29.93 ± 2.15^{aA}	29.33 ± 2.34^{aA}	30.60 ± 3.04^{aA}			
	APSH	26.30 ± 0.72^{aA}	28.00 ± 2.71^{aA}	26.53 ± 3.37^{aA}			
ALT (U/L)	Control	30.27 ± 4.17^{bA}	29.53 ± 3.07^{bA}	21.93 ± 3.37^{aA}	$P < 0.05$	$P = 0.35$	$P = 0.53$
	APSL	22.67 ± 2.69^{aA}	21.13 ± 1.16^{aA}	24.80 ± 4.14^{aA}			
	APSH	22.80 ± 3.10^{aA}	24.53 ± 3.57^{abA}	22.50 ± 2.33^{aA}			
ALP (U/L)	Control	322.07 ± 5.73^{bA}	311.10 ± 25.31^{bA}	374.43 ± 60.62^{bA}	$P < 0.05$	$P = 0.08$	$P = 0.42$
	APSL	239.77 ± 27.36^{aA}	220.13 ± 29.23^{aA}	247.47 ± 39.09^{aA}			
	APSH	357.47 ± 33.04^{bA}	296.27 ± 17.40^{bA}	315.70 ± 43.74^{abA}			
GGT (U/L)	Control	7.10 ± 0.75^{aB}	5.20 ± 0.79^{aA}	6.73 ± 0.80^{aAB}	$P = 0.12$	$P < 0.05$	$P = 0.21$
	APSL	5.90 ± 0.70^{aB}	4.13 ± 0.71^{aA}	6.83 ± 0.85^{aB}			
	APSH	7.50 ± 1.18^{aB}	4.83 ± 0.47^{aA}	8.13 ± 0.78^{aB}			
CK (U/L)	Control	262.60 ± 39.11^{aA}	290.90 ± 24.31^{aA}	234.97 ± 45.24^{aA}	$P = 0.14$	$P < 0.05$	$P = 0.51$
	APSL	286.37 ± 8.52^{aA}	304.50 ± 9.11^{aA}	282.67 ± 33.48^{aA}			
	APSH	273.37 ± 10.65^{aB}	270.87 ± 21.67^{aB}	230.40 ± 13.86^{aA}			
TP (g/L)	Control	50.83 ± 1.50^{aA}	51.40 ± 1.21^{aA}	51.17 ± 1.66^{aA}	$P = 0.32$	$P = 0.11$	$P = 0.92$
	APSL	50.40 ± 2.76^{aA}	51.50 ± 2.60^{aA}	51.03 ± 3.32^{aA}			
	APSH	53.23 ± 1.86^{aA}	54.07 ± 2.65^{aA}	54.27 ± 3.52^{aA}			
ALB (g/L)	Control	25.23 ± 1.65^{aA}	24.60 ± 2.85^{aA}	25.27 ± 2.44^{aA}	$P = 0.23$	$P = 0.38$	$P = 0.21$
	APSL	26.33 ± 1.42^{aA}	27.63 ± 0.31^{aA}	25.90 ± 1.81^{aA}			
	APSH	27.77 ± 2.29^{aA}	28.77 ± 3.31^{aA}	28.33 ± 1.90^{aA}			
GLO (g/L)	Control	25.60 ± 0.46^{aA}	26.80 ± 1.74^{aA}	25.90 ± 0.90^{aA}	$P = 0.42$	$P = 0.50$	$P = 0.50$
	APSL	24.07 ± 1.63^{aA}	23.87 ± 2.66^{aA}	25.13 ± 2.49^{aA}			
	APSH	25.47 ± 1.42^{aA}	25.30 ± 1.21^{aA}	25.93 ± 2.15^{aA}			
A/G	Control	0.99 ± 0.07^{aA}	0.93 ± 0.07^{aA}	0.98 ± 0.13^{aA}	$P = 0.19$	$P = 0.55$	$P = 0.36$
	APSL	1.10 ± 0.06^{aA}	1.17 ± 0.14^{aA}	1.04 ± 0.11^{aA}			
	APSH	1.09 ± 0.13^{aA}	1.14 ± 0.18^{aA}	1.10 ± 0.08^{aA}			
TG (mmol/L)	Control	0.31 ± 0.01^{aA}	0.36 ± 0.04^{aAB}	0.42 ± 0.04^{aB}	$P = 0.28$	$P < 0.01$	$P = 0.29$
	APSL	0.30 ± 0.04^{aA}	0.36 ± 0.05^{aAB}	0.41 ± 0.02^{aB}			
	APSH	0.37 ± 0.06^{aA}	0.35 ± 0.01^{aAB}	0.45 ± 0.05^{aB}			
GLU (mmol/L)	Control	5.80 ± 0.20^{aA}	5.80 ± 0.19^{aA}	5.74 ± 0.16^{aA}	$P = 0.56$	$P = 0.06$	$P = 0.60$
	APSL	5.92 ± 0.39^{aA}	5.70 ± 0.69^{aA}	5.70 ± 0.45^{aA}			
	APSH	5.55 ± 0.29^{aA}	5.44 ± 0.42^{aA}	5.42 ± 0.42^{aA}			
CREA (μmol/L)	Control	35.27 ± 2.03^{aB}	31.97 ± 1.46^{aA}	37.57 ± 0.91^{aB}	$P = 0.63$	$P < 0.01$	$P = 0.70$
	APSL	36.03 ± 3.09^{aB}	29.93 ± 2.75^{aA}	39.07 ± 2.90^{aB}			
	APSH	33.87 ± 1.17^{aAB}	29.67 ± 3.01^{aA}	38.00 ± 4.04^{aB}			
BUN (mmol/L)	Control	5.12 ± 0.32^{aA}	5.61 ± 0.3^{aA}	5.08 ± 0.33^{aA}	$P = 0.71$	$P < 0.01$	$P = 0.71$
	APSL	5.02 ± 0.21^{aA}	6.00 ± 0.50^{aAB}	5.22 ± 0.41^{aAB}			
	APSH	4.95 ± 0.37^{aA}	5.84 ± 0.16^{aB}	5.18 ± 0.24^{aA}			

(Continues)

TABLE 3 (Continued)

Items	Groups	Time			*P* Value		
		Prior to castration	Day 7 after castration	Day 14 after castration	Group	Time	Group × Time
TBIL (μmol/L)	Control	2.55 ± 0.14^{aA}	2.99 ± 0.13^{aB}	2.67 ± 0.18^{aA}	$P = 0.74$	$P < 0.01$	$P = 0.67$
	APSL	2.54 ± 0.14^{aA}	2.87 ± 0.23^{aA}	2.73 ± 0.22^{aA}			
	APSH	2.48 ± 0.18^{aA}	2.80 ± 0.10^{aB}	2.69 ± 0.07^{aAB}			

AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; GGT = glutamyl transpeptidase; CK = creatine kinase; TP = total protein; ALB = albumin; GLO = globulin; A/G = albumin/globulin ratio; TG = triglyceride; GLU = glucose; CREA = creatinine; BUN = blood urea nitrogen; TBIL = total bilirubin. Values are means ± SD (n = 6). Different superscript lowercase letters indicate significant differences in same time among different treatment (Control, APSL, APSH) ($P < 0.05$). Different superscript uppercase letters indicate significant differences in same treatment (Control, APSL, APSH) among different treatment time ($P < 0.05$). Control, Astragalus polysaccharide-supplemented at dose 0 mg/kg diet. APSL, Astragalus polysaccharide-supplemented at dose 400 mg/kg diet. APSH, Astragalus polysaccharide-supplemented at dose 800 mg/kg diet.

TABLE 4 Immune response in serum of beagle dogs supplemented with Astragalus polysaccharide (APS) for 56 days

Items	Groups	Time			*P* Value		
		Prior to castration	Day 7 after castration	Day 14 after castration	Group	Time	Group × Time
CRP (mg/L)	Control	19.22 ± 0.80^{aB}	19.41 ± 1.08^{cB}	15.84 ± 0.78^{bA}	$P < 0.01$	$P < 0.01$	$P < 0.01$
	APSL	19.14 ± 1.19^{aC}	15.52 ± 0.39^{aB}	13.60 ± 1.61^{aA}			
	APSH	17.68 ± 2.25^{aB}	17.69 ± 1.37^{bB}	12.43 ± 0.77^{aA}			
IL-1β (pg/mL)	Control	304.01 ± 50.58^{bA}	589.04 ± 26.09^{cC}	429.01 ± 29.85^{bB}	$P < 0.01$	$P < 0.01$	$P < 0.01$
	APSL	250.55 ± 41.04^{bA}	483.97 ± 31.42^{bC}	312.16 ± 45.85^{aB}			
	APSH	159.97 ± 11.86^{aA}	437.04 ± 33.58^{aB}	445.08 ± 13.89^{bB}			
TNF-α (pg/mL)	Control	43.04 ± 5.73^{aA}	81.20 ± 6.23^{cC}	63.22 ± 3.63^{bB}	$P < 0.01$	$P < 0.01$	$P < 0.01$
	APSL	48.31 ± 5.95^{aA}	68.27 ± 5.22^{bB}	44.24 ± 3.31^{aA}			
	APSH	48.18 ± 3.36^{aB}	54.63 ± 2.127^{aC}	42.81 ± 2.58^{aA}			
IL-10 (pg/ml)	Control	50.26 ± 3.23^{aA}	55.48 ± 5.05^{aB}	67.59 ± 1.96^{aC}	$P < 0.01$	$P < 0.01$	$P = 0.13$
	APSL	56.99 ± 3.81^{bA}	61.42 ± 8.93^{abAB}	67.49 ± 8.84^{aB}			
	APSH	61.72 ± 7.06^{bA}	65.79 ± 8.60^{bA}	75.69 ± 3.28^{bB}			
TGF-β1 (pg/ml)	Control	181.67 ± 13.59^{aC}	131.97 ± 14.40^{aB}	109.66 ± 13.43^{aA}	$P = 0.73$	$P < 0.01$	$P = 0.07$
	APSL	179.09 ± 13.07^{aC}	155.39 ± 24.29^{aB}	99.38 ± 17.09^{aA}			
	APSH	179.00 ± 8.28^{aC}	145.22 ± 19.43^{aB}	112.67 ± 12.86^{aA}			

CRP = C-reactive protein; IL-1β = interleukin 1β; TNF-α = tumor necrosis factor α; IL-10 = interleukin 10; TGF-β1 = transforming growth factor-β1. Values are means ± SD (n = 6). Different superscript lowercase letters indicate significant differences in same time among different treatment (Control, APSL, APSH) ($P < 0.05$). Different superscript uppercase letters indicate significant differences in same treatment (Control, APSL, APSH) among different treatment time ($P < 0.05$). Control, Astragalus polysaccharide-supplemented at dose 0 mg/kg diet. APSL, Astragalus polysaccharide-supplemented at dose 400 mg/kg diet. APSH, Astragalus polysaccharide-supplemented at dose 800 mg/kg diet.

CRP, IL-1β and TNF-α ($P < 0.05$), and there was no significant group × time interaction on the IL-10 and TGF-β1($P > 0.05$). Prior to castration, IL-1β content was the highest in the control group ($P < 0.05$); however, IL-10 content showed the opposite result ($P < 0.05$). On day 7 after castration, the levels of IL-1β and TNF-α were the lowest in the APSH group ($P < 0.05$); the CRP content in the APSL group was lower than that in the control and APSH groups ($P < 0.05$); and IL-10 content was highest in the APSH group ($P < 0.05$). On day 14 after castration, the levels of CRP and TNF-α in the APSL and APSH groups were lower than in the control group ($P < 0.05$), IL-1β was significantly decreased in the APSL group ($P < 0.05$), and IL-10 was significantly increased in the APSH group ($P < 0.05$); In all group, CRP and TNF-α were the highest on day 7 after castration ($P < 0.05$). IL-10 were elevated significantly on day 14 after castration in all treatment. ($P < 0.05$). On the contrary, TGF-β1 was decreased significantly on day 14 after castration ($P < 0.05$).

3.4 | The effect of APS on oxidative stress status

The levels of cortisol, MDA, and PC, and the activities of SOD1, CAT, Se-GPx, and GSH in the serum of dogs are shown in Table 5. Cortisol

TABLE 5 Antioxidant status in serum of beagle dogs supplemented with Astragalus polysaccharide (APS) for 56 days

		Time			*P* Value		
Items	Groups	Prior to castration	Day 7 after castration	Day 14 after castration	Group	Time	Group × Time
Cortisol (ng/dL)	Control	16.76 ± 1.06^{bA}	22.80 ± 3.40^{bB}	21.72 ± 1.49^{bB}	$P < 0.01$	$P < 0.01$	$P < 0.01$
	APSL	11.11 ± 1.08^{aA}	17.67 ± 2.10^{aB}	20.04 ± 3.03^{bB}			
	APSH	10.56 ± 0.81^{aA}	19.80 ± 1.09^{aC}	16.18 ± 1.49^{aB}			
MDA (nmol/mL)	Control	10.00 ± 1.58^{aB}	9.65 ± 0.57^{aB}	6.36 ± 0.68^{aA}	$P = 0.14$	$P < 0.01$	$P = 0.49$
	APSL	10.56 ± 0.84^{aC}	9.24 ± 0.41^{aB}	6.95 ± 1.08^{aA}			
	APSH	10.89 ± 0.25^{aC}	9.49 ± 1.29^{aB}	7.47 ± 0.93^{aA}			
PC (ng/ml)	Control	84.66 ± 4.23^{aB}	82.38 ± 6.39^{bA}	61.06 ± 4.14^{bA}	$P < 0.01$	$P < 0.01$	$P < 0.01$
	APSL	82.83 ± 9.97^{aB}	68.22 ± 2.70^{aA}	57.68 ± 4.38^{abA}			
	APSH	82.49 ± 7.15^{aB}	69.51 ± 11.1^{aA}	53.93 ± 5.12^{aA}			
SOD1 (pg/ml)	Control	312.59 ± 30.73^{aA}	329.57 ± 40.69^{aA}	394.50 ± 53.62^{aB}	$P = 0.23$	$P < 0.01$	$P = 0.74$
	APSL	311.33 ± 45.52^{aA}	377.63 ± 40.71^{bB}	415.94 ± 52.13^{aB}			
	APSH	331.01 ± 59.14^{aA}	360.03 ± 20.77^{abA}	397.28 ± 66.45^{aA}			
CAT (ng/ml)	Control	100.99 ± 8.98^{aA}	112.83 ± 8.39^{aB}	109.66 ± 13.43^{aA}	$P = 0.68$	$P < 0.01$	$P = 0.85$
	APSL	105.58 ± 14.35^{aB}	115.21 ± 9.15^{abB}	99.38 ± 17.09^{aA}			
	APSH	97.46 ± 2.68^{aA}	128.51 ± 15.74^{bC}	112.67 ± 12.86^{aB}			
Se-GPx (ng/ml)	Control	190.17 ± 22.02^{aA}	220.60 ± 15.64^{aB}	182.41 ± 8.34^{aA}	$P < 0.01$	$P < 0.01$	$P = 0.18$
	APSL	219.33 ± 18.28^{aB}	274.51 ± 14.66^{bC}	189.19 ± 9.94^{aA}			
	APSH	191.28 ± 30.89^{aA}	249.28 ± 31.78^{bB}	200.28 ± 39.85^{aA}			
GSH (nmol/ml)	Control	47.42 ± 5.35^{aA}	56.24 ± 6.39^{aB}	43.99 ± 6.98^{aA}	$P < 0.05$	$P < 0.01$	$P = 0.43$
	APSL	52.69 ± 4.34^{aA}	67.07 ± 7.39^{bB}	45.67 ± 3.60^{aA}			
	APSH	47.57 ± 4.01^{aA}	58.84 ± 6.82^{abB}	42.57 ± 2.68^{aA}			

MDA = malondialdehyde; PC = protein carbonyl; SOD1 = Cu/Zn superoxide; Se-GPx = glutathione peroxydase; CAT = catalase; GSH = glutathione. Values are means ± SD (n = 6). Different superscript lowercase letters indicate significant differences in same time among different treatment (Control, APSL, APSH) ($P < 0.05$). Different superscript uppercase letters indicate significant differences in same treatment (Control, APSL, APSH) among different treatment time ($P < 0.05$). Control, Astragalus polysaccharide-supplemented at dose 0 mg/kg diet. APSL, Astragalus polysaccharide-supplemented at dose 400 mg/kg diet. APSH, Astragalus polysaccharide-supplemented at dose 800 mg/kg diet.

and PC showed significant group × time interactions ($P > 0.05$). There were no significant group × time interaction on MDA, SOD1, CAT, Se-GPx, and GSH ($P > 0.05$). Prior to castration, the cortisol contents were lower in the APSH group than in the control group ($P < 0.05$); however, there was no difference between the other oxidative stress status parameters ($P > 0.05$). On day 7 after castration, cortisol content was lower in the APSL and APSH groups than in the control group ($P < 0.05$), and the PC content was lower in the APSH group than in the control group ($P < 0.05$). APSL treatment significantly increased the SOD1 and Se-GPx activities and GSH content compared with control treatment ($P < 0.05$). Moreover, CAT activity was significantly increased in the APSH group ($P < 0.05$). On day 14 after castration, the PC and cortisol levels in the APSH group were lower than those in the control group ($P < 0.05$). Cortisol, CAT, Se-GPx, and GSH were significantly increased on day 7 after castration in all group ($P < 0.05$). The highest content of MDA and PC were observed in all group prior to castration time ($P < 0.05$). While SOD were exhibited significantly increased in Control and APSL group on day 14 after castration ($P < 0.05$).

4 | DISCUSSIONS

4.1 | APS regulates growth and wound healing in beagle dogs

The effects of APS on the growth performance of beagle dogs were revealed in this study. Poor FW and WG were observed in beagle dogs fed high levels of APS, as previously shown in mice (Huang et al., 2017) and rats (Wang et al., 2009). Ahmed and Khan (2006) reported that the decreased growth in animals was due to the increased F:G ratio. Based on the correlation analysis, F:G was negatively correlated with FW and WG, respectively (r1 = −0.588, P1< 0.05; r2 = −1.00, P2< 0.01), implying that poor beagle dog growth may be partly attributed to the improved F:G caused by APS. Castration is a painful and stressful procedure that causes tissue damage and induces host inflammatory responses (Aengwanich et al., 2019). To our knowledge, APS is an herb commonly used in traditional Chinese medicine and is one of the major active ingredients responsible for the biologically active

properties, including immunomodulation, anti-inflammatory, and antioxidant properties (Huang et al., 2017). In this study, the results of wound healing in all castration cases were almost closed, dry, and clean following APS treatment. Unfortunately, no previous studies have investigated the effects of APS on wound healing in beagle dogs. Gilliver et al. (2006) indicated that wound healing is a complex process that is strongly correlated with the inflammatory response, which led us to hypothesize that APS has a positive effect on the inflammatory response in weanling beagle dogs. Thus, we investigated the effects of APS on the inflammatory response in beagle dogs.

4.2 | APS improves the haematological and serum biochemical profiles of beagle dogs

The haematological profile is a critical marker for monitoring animal health and the pathophysiological state (Farag et al., 2019). In this study, all beagle dogs had good or fair body conditions throughout the experimental period. Haematological parameters were within the normal ranges, as found in our previous study (Yang et al., 2020). Interestingly, this study found that haematology parameters, such as WBC, HGB, HCT, MCV, and MCHC, were not significantly influenced by APS. However, APS supplementation decreased HCT levels in beagle dogs after castration. Today, limited data have been reported regarding the effect of APS supplementation on HCT levels in weanling beagle dogs. In this study, the exact mechanisms for the decreased HCT due to APS supplementation are not clear, but could be attributed to a possible decrease in RBC concentration caused by APS supplementation. HCT is the proportion of blood volume that is affected by RBC number and size (Maheswaran et al., 2008). In this study, the levels of RBC were decreased in APS supplementation diet. Correlation analyses revealed that the HCT level was positively related to RBC levels (on day 7 after castration, $r = +0.900$, $P < 0.05$), supporting the hypothesis that the mechanism by which APS decreases the HCT level is related to the downregulation of RBC levels. Furthermore, the effects of APS on RBC contents may be attributed to the decrease in serum iron and the increase in cell apoptosis. Studies have shown that serum iron is positive in RBCs (Kim et al., 2008). In mice, APS supplementation has been reported to reduce serum iron (Ren et al., 2016). In addition, Caspase 3 is a frequently activated cell death, a previous study showed that increased Caspase 3 levels induced apoptosis in animal cells (Feng et al., 2015). In vitro, it has been proved that APS increased the activities of caspase 3 (Cao et al., 2010). Additionally, in this study, APS increased MCH and PLT levels in beagle dogs after castration. The effects of APS on MCH levels may be also explained by a decrement of serum iron. Madhu and Pooja (2015) indicated that an increase in MCH is closely associated with a reduction in blood iron, resulting in decreased oxygen-carrying capacity and eventual stimulation of erythropoiesis. As mentioned earlier, APS supplementation decrease serum iron in mice (Ren et al., 2016). Thus, the increase in MCH levels owing to the APS diet may be a modulation of the oxygen-carrying function, which may be an adaptation to the down-regulation of RBC levels. The increase in the concentration of PLT agrees with the results reported by Denzler et al. (2016), who found that the number of circulating platelets was increased by the administration of APS. PLT provide initial haemostasis in wound healing and produce platelet-derived growth factor (PDGF) and transforming growth factor (TGF), which stimulate the healing of wounds (Grazul-Bilska et al., 2003). In vivo, the administration of APS increased the levels of IL-6 and IL-13 (Denzler et al., 2016), which are involved in PDGF-induced (Roth et al., 1995) and TGF-induced cell proliferation (Booth et al., 2001). Thus, our study supports the notion that APS may induce wound healing through changes in PDGF and TGF; however, the underlying mechanism should be further investigated.

Serum biochemical parameters are another useful tool for diagnosing and investigating the physiological responses of animals to the surrounding environment. Interestingly, this study found that serum biochemical parameters, including AST, GGT, CK, TP, ALB, GLO, A/G, TG, and GLU, were not significantly influenced by APS. However, APS supplementation decreased the ALP and ALT levels in weanling beagle dogs. The significant change in ALP and ALT levels in the current study is in accordance with the results of Farag et al. (2019) and Yan et al. (2009) in rats. The decline in ALP and ALT levels in our study could explain why APS exerts a potential health benefit in beagle dogs through an improvement in liver function. The liver is the main site of protein synthesis and contains ALP and ALT (Casiglia et al., 1993). Damage to the hepatic structure leads to leakage of ALP and ALT into the blood (Abirami et al., 2015). APS has been reported to remarkably ameliorate CCl4-induced cellular boundary loss in hepatocytes (Yan et al., 2009), suggesting that APS supplementation alleviates liver damage (Mahmoodzadeh et al., 2017). Therefore, the decrease in the ALP and ALT content may be attributed to the protection of the structural integrity of hepatocytes by APS supplementation; however, the mechanisms are unknown and should be further investigated.

4.3 | APS improves the immune status of beagle dogs

CRP is regarded as a marker of inflammation, and an increased CRP level is associated with inflammatory bowel disease (Vermeire et al., 2010) and hypertension (Elabd et al., 2016). In this study, APS supplementation decreased CRP levels in weanling beagle dogs, suggesting that APS has positive effects on inflammation status in dogs. This is consistent with the results of studies conducted with mice (Xxa et al., 2020) and rabbits (Zeng et al., 2010). The effects of APS on CRP are most likely due to the production of IL-1β and TNF-α. Studies have shown that the combination of IL-1β and TNF-α can induce CRP production (Vermeire et al., 2010). Our results showed that APS decreased IL-1β and TNF-α levels, showing a similar trend in CRP content. Studies have also indicated that anti-inflammatory cytokines, such as IL-10 and TGF-β1, could counteract the production of pro-inflammatory cytokines, including IL-1β and TNF-α, protecting against the overreaction of inflammatory tissue damage (Feng et al., 2015). This study showed that APS supplementation resulted in a higher IL-10 content

and lower IL-1β and TNF-α content. Rebl et al. (2010) reported that pro-inflammatory cytokine production in terrestrial animals involves the nuclear factor κB (NF-κB) signaling pathway, and a decrease in NF-κB P65 expression reduces IL-1β and TNF-α expression (Neurath and Pettersson, 1997). In vitro, NF-κB p65 was downregulated in the APS group (Huang, 2013). Therefore, APS was found to downregulate IL-1β and TNF-α, which may be closely related to the upregulation of IL-10 and downregulation of the NF-κB signaling pathway. However, these mechanisms are unknown and require further investigation.

4.4 | APS improves antioxidant defenses in beagle dogs

An extensive body of evidence indicates that inflammation is accompanied by oxidative damage (Kruidenier et al., 2003). Plasma cortisol concentrations are often used as indicators of environmental stress in animals (Morton et al., 1995). As animals become more stressed, a physiological release of cortisol occurs (Rostal et al., 2012). In our study, the cortisol content in weanling beagle dogs was lower under APS supplementation, suggesting that the antioxidant status may be improved by APS. Therefore, we next investigated the effects of APS on antioxidant defenses in beagle dogs. The MDA and PC contents are most widely used as convenient markers of oxidative damage to lipids and proteins, respectively (Lin et al., 2013). In our study, APS decreased PC content in the serum of weanling beagle dogs, suggesting that protein oxidative damage was inhibited by APS. Similar results have been documented in rat tilmicosin-induced cardiac injury by targeting oxidative stress (Awad et al., 2018). Oxidative damage in organisms results from excessive ROS production (Turan and Mahmood, 2007), and ROS scavenging ability is correlated with non-enzymatic antioxidants, such as reduced GSH, and antioxidant enzymes such as SOD1, CAT, and Se-GPx (Feng et al., 2015). Therefore, we also determined the effects of APS on GSH content and the activities of SOD1, CAT, and GPX. APS was found to increase the GSH content and SOD1, CAT, and Se-GPx activities in the serum of weanling beagle dogs. Aligning with the present results, Fei et al. (2009) found that APS increased GSH concentration in male Sprague-Dawley rats with chronic liver injury. Li et al. (2012) also reported a marked increase in SOD, CAT, and Se-GPx activity in mouse liver mitochondria after the administration of APS. The positive effects of APS on SOD1, CAT, and Se-GPx activities may be partly explained by the positive effect of APS on the Nrf2-KEAP1 signaling pathway, which plays a key role in the expression of antioxidant enzymes (Szklarz, 2013). The upregulation of Nrf2 transcripts in mice induces SOD1 (Hoshino et al., 2011), CAT (Cho et al., 2005), and Se-GPx (Singh et al., 2006) production. Studies have shown that the administration of APS improves the expression of Nrf2 in the hepatic tissue of adult males (Farag et al., 2019), suggesting that the APS-induced increase in the production of antioxidant enzymes may be partly attributed to the regulation of the Nrf2-KEAP1 signaling pathway; however, the detailed mechanism requires further investigation.

4.5 | Optimum APS supplementation in beagle dogs

APS has been widely used as a safe alternative to antibiotics in feed additives owing to its immunomodulatory, anti-inflammatory, and antioxidant activities (Shahrajabian et al., 2019). In weaned pigs, a quadratic regression analysis indicated that the optimal APS level for an immune response ranges from 324 to 563 mg/kg (Yuan et al., 2006). In LPS-challenged piglets, 800 mg/kg APS in the diet caused lower ALT, AST, IL-1β, and TNF-α levels, and higher SOD activity (Wang et al., 2020). In addition, 800 mg/kg APS caused higher expression of the NF-κB protein in the jejunum of weaned piglets. Zhong et al. (2019) found that a diet supplemented with 200 mg/kg APS can enhance serum interleukin-2 content and increase the digestion and absorption capacity of the intestinal tract of silver foxes. Thus, the experimental diets were supplemented with 0, 400, and 800 mg/kg APS. The results of this study demonstrate that supplementation with APS at a dose of 400 mg/kg had no significant effects on the growth of growing beagles, but could have positive effects on wound healing in weanling beagle dogs by improving the haematological parameters, serum biochemical profile, and immune and antioxidant status. Therefore, the optimum APS concentration for supplementation was identified as 400 mg/kg.

5 | CONCLUSIONS

In conclusion, this study demonstrated that supplementing weanling beagle dogs with optimum APS could positively affect wound healing by improving their haematological profile (decreased RBC and HCT content, increased MCH and PLT levels), serum biochemical parameters (decreased ALP and ALT content), immune status (decreased CRP, IL-1β, and TNF-α levels; increased IL-10 content), and antioxidant defense (decreased cortisol and PC content; increased GSH content, and SOD1, CAT, and Se-GPx activities). However, the detailed mechanism whereby APS regulates these changes requires further investigation. In addition, the results of this study suggest that 400 mg/kg diet is the optimum APS dose for beagle dogs.

AUTHOR CONTRIBUTIONS

Jian-Bo Luo, Han Dong, and Lei Zhang designed research; Min Fu, Yang Hong, Xin-Yin Du, Guo-Qiang Cheng, and Jie-Ying Xia conducted research; Jianbo Luo, Han Dong, and Min Fu analyzed data; Jian-Bo Luo, Han Dong, and Min Fu wrote the paper; Jianbo Luo, Han Dong, Min Fu, and Lei Zhang revised the paper.

CONFLICT OF INTEREST

We declare that we have no financial or personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any

nature or kind in any product, service, and/or company that could be construed as influencing the content of this paper.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Animal Care Advisory Committee of the Institute of Laboratory Animal Sciences, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital (license number: AE2020009). Animal Care Advisory Committee of the Institute of Laboratory Animal Sciences, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital specifically approved this study (license number: AE2020009). The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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PEER REVIEW

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