Effects of Sea Buckthorn Pomace on Growth Performance, Organ Indices, Serum Biochemical Indices, and Intramuscular Fatty Acid Composition in Fattening Lambs: Postprint

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Abstract

This study aimed to investigate the effects of dietary seabuckthorn pomace supplementation on growth performance, organ indices, serum biochemical indices, and intramuscular fatty acid composition in fattening lambs. Twenty-four 3-month-old Dorper × Small-tailed Han crossbred male lambs [(25$±$1) kg] were randomly allocated to 4 groups with 6 lambs per group. Each group was fed experimental diets containing 0 (control), 10%, 20%, and 30% seabuckthorn pomace, respectively. The trial lasted 50 days. The results showed: 1) The pre-slaughter live weight of lambs in the 10% and 20% groups was significantly higher than that in the control and 30% groups ($P<0.05$), the average daily gain of lambs in the 10% group was significantly higher than that in other groups ($P<0.05$), the average daily feed intake of lambs in the 20% group was significantly higher than that in other groups ($P<0.05$), and the feed conversion ratio of lambs in the 10% group was significantly lower than that in other groups ($P<0.05$). 2) The liver and spleen indices of lambs in the 30% group were significantly higher than those in other groups ($P<0.05$), while the reticulum index was significantly lower than that in other groups ($P<0.05$). The large intestine and small intestine indices of lambs in the 10%, 20%, and 30% groups were significantly higher than those in the control group ($P<0.05$). 3) The serum high-density lipoprotein (HDL) content of lambs in the 30% group was significantly higher than that in other groups ($P<0.05$), the atherosclerosis index (AI) of lambs in the 10%, 20%, and 30% groups was significantly lower than that in the control group ($P<0.05$), the serum total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities of lambs in the 10%, 20%, and 30% groups were significantly higher than those in the control group ($P<0.05$), and the serum aspartate aminotransferase (AST),

alanine aminotransferase (ALT), lactate dehydrogenase (LDH) activities and creatinine (CRE) and urea nitrogen (UN) contents of lambs in the 30% group were significantly higher than those in other groups (P<0.05). 4) The proportions of linoleic acid, elaidic acid, arachidonic acid, total polyunsaturated fatty acids (ΣMUFA), and total unsaturated fatty acids (ΣUFA) in the longissimus dorsi muscle of lambs in the 10%, 20%, and 30% groups were significantly higher than those in the control group (P<0.05), while the proportions of oleic acid, total saturated fatty acids (ΣSFA), and total monounsaturated fatty acids (ΣPUFA) were significantly lower than those in the control group (P<0.05). In conclusion, dietary seabuckthorn pomace supplementation in fattening lambs is beneficial for their growth and development and improves growth performance, with an appropriate supplementation level of 10-20%.

Full Text

Effects of Sea Buckthorn Pomace on Growth Performance, Organ Indexes, Serum Biochemical Parameters, and Intramuscular Fatty Acid Composition of Fattening Lambs

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Abstract: This study investigated the effects of dietary sea buckthorn pomace on growth performance, organ indexes, serum biochemical parameters, and intramuscular fatty acid composition of fattening lambs. Twenty-four 3-month-old Dorper × Small-tailed Han crossbred ram lambs [(25$±$1) kg] were randomly allocated to four groups (n=6). The groups were fed experimental diets containing 0% (control), 10%, 20%, and 30% sea buckthorn pomace for 50 days. The results showed: (1) Lambs in the 10% and 20% groups had significantly higher pre-slaughter live weight compared to the control and 30% groups (P<0.05). The 10% group exhibited the highest average daily gain, significantly surpassing all other groups (P<0.05). The 20% group showed the greatest average daily feed intake, significantly higher than other groups (P<0.05). The 10% group had the lowest feed-to-gain ratio, significantly lower than other groups (P<0.05). (2) The 30% group displayed significantly higher liver and spleen indexes (P<0.05) but significantly lower reticulum index compared to other groups (P<0.05). The large intestine and small intestine indexes in the 10%, 20%, and 30% groups were significantly higher than the control group (P<0.05). (3) Serum high-density lipoprotein (HDL) content in the 30% group was significantly higher than other groups (P<0.05). The atherosclerosis index (AI) in the 10%, 20%, and 30% groups was significantly lower than the control group (P<0.05). Serum total antioxidant capacity (T-AOC) and activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in the 10%, 20%, and 30% groups were significantly higher than the control group (P<0.05). The 30% group showed significantly elevated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) activities, and creatinine (CRE) and urea nitrogen (UN) contents compared to other groups (P<0.05). (4) The proportions of linoleic acid, elaidic acid, arachidonic acid, total monounsaturated fatty acids (ΣMUFA), and total unsaturated fatty acids (ΣUFA) in the longissimus dorsi muscle of lambs in the 10%, 20%, and 30% groups were significantly higher than the control group (P<0.05), while oleic acid, total saturated fatty acids (ΣSFA), and total polyunsaturated fatty acids (ΣPUFA) proportions were significantly lower (P<0.05). In conclusion, dietary supplementation with sea buckthorn pomace benefits lamb growth and development, with an optimal inclusion level of 10%–20%.

Keywords: sea buckthorn pomace; fattening lambs; growth performance; serum biochemical parameters; intramuscular fatty acids

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Sea buckthorn (*Hippophae rhamnoides* Linn.) is a deciduous shrub whose fruits contain numerous nutrients and bioactive compounds, including vitamins, carotenoids, minerals, flavonoids, sterols, polyphenols, and essential oils, offering extensive nutritional and medicinal value. Research has demonstrated that sea buckthorn juice consumption can increase plasma high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels while reducing low-density lipoprotein (LDL) oxidation susceptibility in humans. Flavonoids in sea buckthorn can inhibit preadipocyte differentiation, thereby reducing intracellular lipid accumulation and exerting regulatory effects on lipid metabolism while significantly ameliorating oxidative stress.

As a high-quality livestock product, mutton is favored for its high lean meat content, low fat, and low cholesterol. However, mutton contains relatively high levels of saturated fatty acids, which are detrimental to human health. In China's intensive sheep production systems, animals are typically housed indoors at high stocking densities, which can induce oxidative stress, leading to reduced growth performance and increased disease incidence. Sea buckthorn pomace, the residue remaining after sea buckthorn fruit juice extraction, retains substantial nutritional value and represents a potential feed resource. While widely applied in monogastric animal production, research on its use in ruminants remains scarce. Given its high nutritional value and the abundant sea buckthorn pomace resources in Shanxi Province, its application in mutton sheep production could offer significant promotional value. This study utilized Dorper × Small-tailed Han crossbred ram lambs to evaluate different dietary levels of sea buckthorn pomace, examining its effects on growth performance, organ indexes, serum biochemical parameters, and intramuscular fatty acid composition to provide a basis for its application in sheep production.

1.1 Experimental Design

The sea buckthorn pomace used in this experiment was provided by a sea buckthorn beverage factory in Youyu County, Shuozhou City, Shanxi Province. The raw sea buckthorn material was sourced locally from Youyu and consisted of the Chinese sea buckthorn variety (*H. r. subsp. sinensis*). The nutrient levels of the sea buckthorn pomace are presented in Table 1 .

The basal diet was formulated according to the NRC (2007) nutrient requirements for 25 kg ram lambs with an average daily gain of 200 g. The other three diets were supplemented with 10%, 20%, and 30% sea buckthorn pomace, respectively, while adjusting the proportions of corn, soybean meal, and roughage to maintain similar energy and protein levels to the basal diet. All experimental diets were processed as total mixed pellets. The composition and nutrient levels of the experimental diets are shown in Table 2 .

Twenty-four 3-month-old Dorper × Small-tailed Han crossbred ram lambs with similar body weight [(25$±$1) kg] and good health condition were selected and randomly divided into four groups (n=6). Each group was fed experimental diets containing 0% (control), 10%, 20%, and 30% sea buckthorn pomace, respectively.

1.2 Animal Management

The feeding trial was conducted from May to August 2015 at Hongyu Animal Husbandry Co., Ltd. in Youyu County, Shanxi Province. The trial lasted 65 days, including a 15-day preliminary period and a 50-day formal experimental period. Prior to the experiment, the sheep pens were disinfected and cleaned, and the experimental animals were inspected and quarantined. Deworming and group allocation were performed during the preliminary period. During the formal period, lambs were fed twice daily at 08:00 and 18:00 with ad libitum access to feed and water.

1.3 Sample Collection and Measurements

All experimental lambs were weighed on an empty stomach on day 1 and the final day of the formal period. Daily feed intake, initial body weight, and preslaughter live weight were recorded to calculate average daily gain, average daily feed intake, and feed-to-gain ratio.

On the final day of the experiment, all lambs were deprived of water and feed at 16:00. The following morning at 08:00, after weighing, blood samples (10 mL) were collected from each lamb into clean centrifuge tubes, allowed to stand at room temperature for 1 hour, then centrifuged at 3,000 r/min for 10 minutes. Serum was separated and aliquoted into 1.5 mL EP tubes and stored frozen. Serum biochemical parameters were determined by Beijing Huaying Biotechnology Research Institute.

For slaughter sampling, the left longissimus dorsi muscle was collected and stored in liquid nitrogen. The heart, liver, spleen, lungs, kidneys, and other organs were separated, weighed, and recorded. Digestive organs including the rumen, reticulum, omasum, abomasum, large intestine, and small intestine were cleaned and weighed. Organ indexes were calculated as: Organ index (mg/kg) = organ weight (mg) / live body weight (kg). Thirty-six fatty acid components in the longissimus dorsi muscle were determined by Qingdao Kebiao Testing Company.

1.4 Statistical Analysis

Data were initially processed using Excel 2013, then subjected to one-way ANOVA using SPSS 22.0 statistical software, followed by Duncan' s multiple comparison test. Results are expressed as "mean ± standard deviation."

2.1 Effects of Sea Buckthorn Pomace on Growth Performance and Organ Indexes

As shown in Table 3 , no significant differences were observed in initial body weight among groups ($P>0.05$). At the end of the experiment, lambs in the 10% and 20% groups exhibited significantly higher pre-slaughter live weight compared to the control and 30% groups ($P<0.05$). The 10% group achieved the highest average daily gain, significantly exceeding all other groups ($P<0.05$), while the 20% group was significantly higher than the control and 30% groups ($P<0.05$). Average daily feed intake displayed a trend of initially increasing then decreasing with rising dietary sea buckthorn pomace levels, peaking in the 20% group, which was significantly higher than other groups ($P<0.05$), and lowest in the 30% group, which was significantly lower than other groups ($P<0.05$). The 10% group showed the lowest feed-to-gain ratio, significantly lower than other groups ($P<0.05$), whereas the 30% group had the highest ratio, significantly higher than other groups ($P<0.05$).

Table 4 reveals that no significant differences existed among groups in heart, lung, or spleen indexes ($P>0.05$). However, the 30% group demonstrated significantly higher liver and kidney indexes compared to other groups ($P<0.05$). No significant differences were observed in rumen, omasum, or abomasum indexes among groups ($P>0.05$). The 20% group exhibited the highest reticulum index, significantly greater than other groups ($P<0.05$), while the 30% group showed the lowest reticulum index, significantly lower than other groups ($P<0.05$). The large intestine and small intestine indexes in the 10%, 20%, and 30% groups were significantly higher than those in the control group ($P<0.05$).

2.2 Effects of Sea Buckthorn Pomace on Serum Biochemical Parameters

Table 5 shows that no significant differences were detected among groups in serum total protein (TP), albumin (ALB), total cholesterol (TC), triglycerides

(TG), low-density lipoprotein (LDL), glucose (GLU), calcium (Ca), or phosphorus (P) levels ($P>0.05$). However, serum high-density lipoprotein (HDL) content in the 30% group was significantly higher than other groups ($P<0.05$). The atherosclerosis index (AI) decreased progressively with increasing dietary sea buckthorn pomace levels, with the 30% group significantly lower than other groups ($P<0.05$). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) activities in the 30% group were significantly higher than other groups ($P<0.05$). No significant differences were observed among groups in serum alkaline phosphatase (ALP), gamma-glutamyl transferase (G-GT), catalase (CAT), or creatine kinase (CK) activities ($P>0.05$). Serum total antioxidant capacity (T-AOC) and activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in the 10%, 20%, and 30% groups were significantly higher than the control group ($P<0.05$). Serum creatinine (CRE) and urea nitrogen (UN) contents in the 30% group were significantly higher than other groups ($P<0.05$).

2.3 Effects of Sea Buckthorn Pomace on Intramuscular Fatty Acid Composition in Longissimus Dorsi

As presented in Table 6 , the proportions of palmitoleic acid (C16:1) in the 20% and 30% groups were significantly higher than the other two groups ($P<0.05$). The 10%, 20%, and 30% groups exhibited significantly lower oleic acid (C18:1N9C) proportions but significantly higher elaidic acid (C18:1N9T) proportions compared to the control group ($P<0.05$). Linoleic acid (C18:2N6C) and arachidonic acid (C20:4N6) proportions were significantly higher in the 10%, 20%, and 30% groups than the control group ($P<0.05$). Total saturated fatty acids (ΣSFA) and total polyunsaturated fatty acids (ΣPUFA) proportions were significantly lower in the 10%, 20%, and 30% groups compared to the control group ($P<0.05$), whereas total monounsaturated fatty acids (ΣMUFA), total unsaturated fatty acids (ΣUFA) proportions, and the unsaturation index were significantly higher ($P<0.05$).

3.1 Effects of Sea Buckthorn Pomace on Growth Performance and Organ Development

Previous studies have demonstrated significant effects of sea buckthorn pomace on monogastric animal growth, though reports on its application in ruminants remain scarce. In this experiment, dietary supplementation with 10% and 20% sea buckthorn pomace significantly improved average daily gain, while 30% supplementation significantly reduced average daily gain, indicating that moderate inclusion promotes lamb weight gain whereas excessive levels impair growth performance. These findings align with Nuernberg et al.' s results from sea buckthorn pomace supplementation in pig fattening trials. The 10% group achieved the lowest feed-to-gain ratio, representing the highest economic efficiency.

Sea buckthorn exhibits significant hepatoprotective effects, primarily attributed to its fruit oil content. Research has shown that sea buckthorn fruit protects liver cells in rats fed high-fat diets and provides preventive and protective effects against lipopolysaccharide (LPS)/D-galactosamine (D-GalN)-induced liver injury. In intensive lamb fattening, high feed intake and nutrient provision promote rapid weight gain, placing considerable stress on the digestive system, circulatory system, and internal organs. In this study, lambs in the 10% and 20% groups showed improved weight gain without significant adverse effects on serum liver and kidney indicators or hepatic and renal organ indexes.

Sea buckthorn positively affects the gastrointestinal environment. Li et al. found that active antioxidant substances in sea buckthorn seed oil significantly improved intestinal morphology in mice with enteritis. Shen et al. demonstrated that bioactive compounds in sea buckthorn can be effectively absorbed in specific small intestinal segments, thereby improving the intestinal environment. Du et al. reported that dietary sea buckthorn pomace significantly affected intestinal environment in laying hens, promoting beneficial bacteria growth while inhibiting harmful bacteria. Zuo et al. observed that sea buckthorn extract supplementation significantly increased digestive enzyme activities and villus height in the jejunum and ileum of weaned piglets. Jing et al. confirmed that sea buckthorn pulp and seed oils effectively repaired and protected against radiation-induced acute intestinal injury. In this experiment, the significantly higher large intestine and small intestine indexes in the 10%, 20%, and 30% groups indicate positive effects of dietary sea buckthorn pomace on intestinal development.

3.2 Effects of Sea Buckthorn Pomace on Serum Biochemical Parameters

Aspartate aminotransferase and alanine aminotransferase are commonly used indicators of liver function. Under normal physiological conditions, ALT is primarily distributed in hepatocyte cytoplasm, while AST is located in both hepatocyte cytoplasm and mitochondria. Severe hepatic necrosis or damage causes increased serum AST and ALT activities. In this study, the 30% group exhibited significantly higher serum AST and ALT activities than other groups, suggesting that 30% sea buckthorn pomace inclusion may cause liver injury.

Lactate dehydrogenase is a glycolytic enzyme present in the cytoplasm of all tissue cells, with particularly high concentrations in the kidneys. Elevated LDH activity indicates liver or kidney damage. Creatinine is a small molecule filtered by glomeruli with minimal tubular reabsorption; daily creatinine production is almost entirely excreted in urine and is generally unaffected by urine volume. During renal insufficiency, accumulated creatinine becomes a harmful toxin affecting digestive and respiratory metabolism. Serum urea nitrogen, the end product of protein metabolism, is normally excreted by glomeruli and accumulates only when renal dysfunction occurs, causing further kidney damage. In this experiment, the 30% group showed elevated LDH activity and CRE and UN contents, while other groups did not differ significantly from the control, indicating that 30% sea buckthorn pomace inclusion may adversely affect kidney function.

Furthermore, the 30% group exhibited significantly lower weight gain and feed intake, along with larger liver and kidney indexes. When organ damage impairs metabolic function, compensatory organ enlargement occurs, increasing organ indexes. Therefore, we hypothesize that 30% sea buckthorn pomace inclusion caused a certain degree of liver and kidney damage, affecting growth and development. No previous reports have documented sea buckthorn pomace-induced liver and kidney damage from excessive intake.

Total antioxidant capacity, peroxidase, glutathione peroxidase, and superoxide dismutase are direct indicators of antioxidant capacity. This experiment demonstrated that sea buckthorn pomace increased serum T-AOC, GSH-Px, and SOD activities in sheep, confirming its strong antioxidant properties, consistent with previous reports. Rashid et al. demonstrated that sea buckthorn is a non-toxic plant-based antioxidant supplement. Hu et al. found that sea buckthorn pomace ethanol extract significantly increased serum T-AOC, GSH-Px, and SOD activities in mice. Jiao et al. also confirmed through mouse experiments that sea buckthorn flavonoids enhanced serum total antioxidant capacity. Improved antioxidant capacity may indirectly contribute to enhanced growth performance.

Ma et al. reported that sea buckthorn flavonoids regulate intramuscular fat deposition in broilers by modulating insulin and adiponectin levels to alter LDL and TG contents. In this experiment, the 30% group showed higher serum HDL content, though serum total cholesterol did not differ significantly among groups. The AI in the 10%, 20%, and 30% groups was significantly lower than the control group. AI is an internationally recognized medical indicator of atherosclerosis severity; higher AI values indicate greater atherosclerotic risk. The significantly lower AI in sea buckthorn-supplemented groups suggests a protective role against atherosclerosis.

3.3 Effects of Sea Buckthorn Pomace on Intramuscular Fatty Acid Composition

Several studies have reported on sea buckthorn pomace effects on muscle fatty acid profiles. Wang et al. demonstrated that sea buckthorn flavonoids significantly promoted unsaturated fatty acid formation in mice. Wang confirmed that sea buckthorn extract promoted fat decomposition and reduced intramuscular fatty acid content in broilers. Nuernberg et al. verified that sea buckthorn pomace increased saturated fatty acid proportions and significantly elevated oleic acid levels in pigs. In contrast, this experiment found that oleic acid proportions were significantly lower in the 10%, 20%, and 30% groups compared to the control, while linoleic acid proportions were significantly higher and saturated fatty acid proportions decreased. These discrepancies may be attributed to the unique rumen structure of ruminants.

Sea buckthorn pomace contains high fat content, with dietary fatty acids primarily in triglyceride form. These are hydrolyzed by esterases secreted by rumen microorganisms such as *Anaerovibrio lipolytica* into glycerol and free fatty acids. The liberated fatty acids are predominantly converted to stearic acid by *Butyrivibrio* bacteria, while rumen protozoa and other microorganisms hydrogenate unsaturated fatty acids to varying degrees, transforming most dietary unsaturated fatty acids into saturated forms. Monogastric animals lack this ruminal hydrogenation process before gastric digestion. Additionally, flavonoid compounds such as rutin and quercetin in sea buckthorn pomace influence lipid metabolism, thereby affecting fatty acid synthesis. Wang reported that sea buckthorn flavonoids reduced fat accumulation and enhanced antioxidant capacity in mice. Zhang et al. indicated that sea buckthorn extract regulated serum leptin content and adipose leptin mRNA expression, thereby affecting fat synthesis and animal growth performance. Current research on sea buckthorn' s effects on fatty acid synthesis in ruminants is limited, and the underlying mechanisms require further investigation.

With increasing consumer demands for meat quality, enhancing unsaturated fatty acid content has become a research priority. In this experiment, sea buckthorn pomace supplementation significantly reduced saturated fatty acid proportions while increasing unsaturated fatty acids, including both monounsaturated and polyunsaturated fatty acids. Higher unsaturated fatty acid proportions in meat benefit consumer health. Notably, monounsaturated fatty acid content decreased while polyunsaturated fatty acid content increased in supplemented groups. Monounsaturated fatty acids significantly regulate blood pressure, blood lipids, and cholesterol, while polyunsaturated fatty acids importantly influence cardiovascular disease, obesity, and visual development.

The 10%, 20%, and 30% groups showed significantly reduced saturated fatty acids like oleic acid and increased unsaturated fatty acids such as linoleic acid, palmitoleic acid, and arachidonic acid. Cholesterol must combine with linoleic acid before participating in normal human metabolism; linoleic acid deficiency leads to saturated fatty acid-cholesterol complexes that deposit on blood vessel walls, causing metabolic disorders and atherosclerotic cardiovascular disease. Arachidonic acid is an essential fatty acid and crucial neural tissue component with extensive physiological functions, including lipid-lowering, platelet aggregation inhibition, anti-inflammatory, anti-cancer, anti-lipid oxidation, and promotion of brain tissue development. Therefore, from a health perspective, higher contents of unsaturated fatty acids like linoleic and arachidonic acid indicate superior meat quality. Considering all fatty acid proportions, the 20% inclusion level proved optimal.

Conclusions:

1. Dietary supplementation with 10%-20% sea buckthorn pomace can improve growth performance, promote development of vital organs such as the reticulum, large intestine, and small intestine, and enhance serum antioxidant capacity in fattening lambs.

2. Dietary sea buckthorn pomace affects intramuscular fatty acid composition, increasing unsaturated fatty acid proportions while decreasing satu-

rated fatty acid proportions.

3. Sea buckthorn inclusion levels exceeding 30% may impair growth performance and cause a certain degree of liver and kidney damage in fattening lambs.
4. Under the conditions of this study, the optimal dietary inclusion level of sea buckthorn pomace is 10%-20%.

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Note: Figure translations are in progress. See original paper for figures.