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Reducing the dietary starch:protein ratios in low-protein diets enhanced the growth performance of goslings from 1 to 28 days of age

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ABSTRACT

The current study aimed to explore the suitable starch: protein ratios under different dietary protein levels for goslings. A total of 360 male 1-day-old Jiangnan White goslings were randomly divided into 6 groups with six replicates containing ten goslings each. The experimental design consisted of a 3 × 2 factorial array of treatments. Three protein levels (18%, 16%, 14%) and two starch: protein ratio (S: P ratio) types (standard, reduced) were formulated. The results showed that: reducing the S: P ratio at the same dietary protein level increased weight gain (WG), average daily gain (ADG), and average daily feed intake (ADFI) of goslings ($P < 0.05$). Lowering the protein level increased feed-to- gain ratio (F/G) at the same dietary S: P ratio type. Both decreasing dietary protein levels and reducing S: P resulted in an increase ($P < 0.05$) in the serum albumin (ALB) content of goslings. Protein at 18% level, minimized serum total cholesterol (TC) in goslings. Reducing the dietary S: P ratio elevated serum lipid concentration. In reduced S: P ratio diets, serum Leucine (Leu) decreased and Threonine (Thr) concentration increased. The reduction in dietary protein level and S: P ratio significantly affected the amino acid composition of muscles. The varied levels of protein and S: P ratio types interacted to influence the starch digestibility of distal jejunum. In addition, the reduced S: P ratio attenuates α-amylase activity of jejunal chyme. Moreover, *SGLT1* and *GLUT2* genes expression were generally down-regulated, and *SLC7A5* gene expression was up-regulated in reduced S: P ratio groups. In summary, the diet with 14% protein level, and 2.97 starch: protein ratio is recommended to use in gosling's growth phase of 1 to 28 days.

Introduction

Currently, the shortage of high-quality raw protein sources poses a challenge to the feed industry, and their increased prices reduced the economic efficiency of farming (Mottet et al., 2017). Although, high-protein diets cause more nitrogen deposition, leading to environmental pollution (Hernandez et al., 2012). Anyhow, soybean meal is the most essential and economic protein source for poultry production, making up at least 25% of the feed. As a result, developing low-protein diets is imperative. Nowadays, The basic idea behind the development of low-protein diets is to reduce the amount of soybean meal and add exogenous nutrients to the diet, such as amino acids, proteases, etc. (Selle et al., 2020; Wang et al., 2020). Liang et al. (2023) found that lowering the dietary protein level from 18.55% to 15.55% and supplementing with 12 essential amino acids, did not negatively affect growth performance of 1-28 d goslings, but their nitrogen excretion reduced by 19.71%. The result indicated that the low protein level in the diet of goslings that can meet the growth and development of goslings may not only be 15.55%, but might be further reduced. In addition, several experiments on broilers (Son et al., 2003; Kobayashi et al., 2012) and ducks (Xie et al., 2016; Jiang et al., 2017) have sufficiently demonstrated the feasibility of low-protein diets.

When dietary soybean meal content is reduced, the grain (corn or wheat) inclusions increase in the diet, which inevitably increases the starch: protein ratio. It definitely affects the digestive dynamics of starch and protein causing more fat deposition (Liu et al., 2017). There were similar findings in the two broiler experiments as Capping the S: P ratio from 1.97 to 1.63 in diets (CP, 19.75%) for Ross 308 chicks from 7-35 d increased weight gain by 10.37% (Greenhalgh et al., 2020). Moreover, Limiting dietary S: P ratios improved weight gain, and the effect

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Table 1
Outline of dietary treatments.

Diet	Description CP/starch: protein ratio	CP level (%)	Starch: protein ratio
A	High protein/standard	18	2.27
B	Medium protein/standard	16	2.70
C	Low protein/standard	14	3.26
D	High protein/reduced	18	2.06
E	Medium protein/reduced	16	2.47
F	Low protein/reduced	14	2.97

Note: Starch: protein ratio is calculated value.

Table 2
Composition and nutrient levels of experimental diets for 1-28 d goslings.

Items	Treatments[1]					
	A	B	C	D	E	F
Ingredients, %						
Corn	62.31	65.35	68.56	53.95	57.30	60.95
Soybean meal	29.27	23.28	17.30	28.62	22.38	16.94
Wheat bran	2.57	4.88	7.02	9.74	12.64	12.87
Rice hull	2.16	2.21	2.24	2.06	1.44	2.45
Limestone	0.90	0.95	1.00	0.94	0.98	1.01
$CaHPO_4$	1.27	1.27	1.27	1.22	1.21	1.22
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Soy oil	0.00	0.00	0.00	1.90	1.95	1.95
L-lysine HCl	0.03	0.17	0.31	0.03	0.17	0.30
DL-methionine	0.19	0.22	0.25	0.20	0.22	0.25
L-leucine	0.00	0.15	0.31	0.04	0.19	0.34
L-threonine	0.00	0.08	0.17	0.00	0.09	0.17
L-tryptophan	0.00	0.03	0.06	0.00	0.03	0.05
L-valine	0.00	0.11	0.21	0.00	0.10	0.20
Permix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient levels[3], %						
Metabolizable energy (MJ/Kg)	11.55	11.49	11.45	11.43	11.41	11.41
Crude protein	18.11	16.08	13.99	18.07	16.06	14.07
Starch	41.10	43.28	45.53	37.19	39.67	41.84
Starch: protein ratio	2.27	2.70	3.26	2.06	2.47	2.97
Crude fiber	4.28	4.17	4.04	4.47	4.10	4.32
Calcium	0.82	0.82	0.82	0.82	0.81	0.82
Available phosphorus	0.38	0.38	0.38	0.38	0.38	0.38
Lysine	0.97	0.99	1.00	0.95	0.96	0.99
Methionine	0.48	0.47	0.49	0.48	0.46	0.47
Leucine	1.59	1.60	1.61	1.58	1.62	1.60
Threonine	0.68	0.69	0.65	0.66	0.67	0.67
Tryptophan	0.20	0.22	0.23	0.19	0.21	0.21
Valine	0.85	0.87	0.86	0.85	0.88	0.86

[1] A: High protein, Standard S: P ratio; B: Medium protein, Standard S: P ratio; C: Low protein, Standard S: P ratio; D: High protein, Reduced S: P ratio; E: Medium protein, Reduced S: P ratio; F: Low protein, Reduced S: P ratio.
[2] The premix supplied per kilogram of diet: vitamin A, 12000 IU; vitamin D_3, 4000 IU; vitamin E, 28 mg; vitamin K_3, 1.5 mg; vitamin B_1, 0.9 mg; vitamin B_2, 8 mg; vitamin B_6, 3.2 mg; vitamin B_{12}, 0.01 mg; nicotinic acid, 45 mg; pantothenic acid, 11 mg; folic acid, 0.65 mg; choline chloride, 0.45g; biotin, 0.05 mg; Fe, 60 mg; Cu, 10 mg; Mn, 95 mg; Zn, 90 mg; I, 0.5 mg; Se, 0.3 mg.
[3] Analyzed values except for Metabolizable energy, calcium and available phosphorus.

was most pronounced at a 17.5% protein level (Greenhalgh et al., 2022). The following two experiments validate that reducing starch content (S: P ratio) in low-protein diets suits growth performance. The success of reducing the dietary S: P ratio might be associated with the decreased starch digestibility and slower disappearance rate of starch in the small intestine.

Excess starch in low-protein diets breaks down more glucose in the intestine, and overloaded glucose disrupts amino acid absorption (Selle et al., 2019). As a result, both glucose and amino acids can break down to provide energy for the intestines. Generally, glucose contains a higher energy supply efficiency than amino acids. The digestive dynamics of starch and protein is, to control the decomposition of glucose and amino acids so that more glucose is available to supply energy in the intestines. So, more amino acids pass through the intestinal mucosa and enter the portal vein to synthesize body proteins. Competitive uptake of glucose and amino acids may occur when both are absorbed along the small intestine (Murer et al., 1975). This may be due to competition between glucose and amino acids uptake via their respective Na^+-dependent transporters in intestine (Macelline et al., 2020). Most of the glucose is absorbed from the intestinal lumen with Na^+ through the SGLT1 transporter, while the GLUT2 transporter transfers glucose from the basolateral channel of enterocytes into the portal circulation (Daniel et al., 2015).

The primary purpose of the present study was to explore the effects of reducing the dietary starch: protein ratio at different protein levels on the growth performance and intestinal starch digestion of goslings and to preliminarily explore whether this strategy will cause competition for intestinal uptakes between glucose and amino acids or not.

Table 3
Primers used in real-time quantitative PCR.

Gene name	Primer sequence (5'-3')	Product size (bp)	Gene Bank NO./ Reference
SGLT-1	F: CTTATGCCAAATGGTCTGCGAG R: CATAAATGCCCTTCCAGCCAAC	174	MG925328.1
GLUT-2	F: GATGGTCCAGATATCCCAGCAG R: AATGGTTGCATAAACGGGTTGG	106	MG925329.1
SLC7A5	F: GCTTCTCACTCCTGTGCCAT R: TCACCTTGATGGGCCTTTCC	188	XM_013197556.2
SLC6A14	F: TCACCTACCAGAACGGTGGA R: ACGCCCACTCCTTGAAACAA	163	XM_013183397.2
SLC38A1	F: AGCTTGGTGAGCAGGTCTTT R: TGGCAGAAGGCAGCTCATTT	123	XM_013198961.2
β-action	F: GAAATCGTGCGTGACATCAA R: GCAGGACTCCATACCCAAGA	198	XM_013174886.1

Materials and methods

All animal care and experimental procedures in the study were performed according to the Regulations for the Administration of Affairs Concerning Experimental Animals of the People's Republic of China and approved by the Animal Care and Use Committee of Yangzhou University Yangzhou, China (SYXK (Su) IACUC 2021-0036).

Experimental diets and design

Total 360 male 1-day-old Jiangnan White goslings, supplied by the Jiangsu Lihua Animal Husbandry Co., LTD (Changzhou, China), were randomly divided into six groups with six replicates and each replicate containing ten goslings. The experiment designed as 3 × 2 factorial array of dietary treatments, as outlined in Table 1. Three protein levels (18%, 16%, 14%) and two starch: protein ratio types (standard, reduced) were formulated. There is a standard S: P ratio for every protein level., and every standard ratio was lowered by 9% to obtain the corresponding reduced S: P ratio. All the diets were formulated to the same energy density (AME=11.5 MJ/kg). The dietary composition and nutrient levels were shown in Table 2. The group with a protein level of 18% and an S: P ratio of 2.27 was the control group of this experiment, which can basically meet the growth and physiological needs of goslings.

The goslings were raised on plastic nets for the whole experiment period (single pen area: 1.9 m × 1.5 m, 2.85 m^2), and the stocking density of goslings from 1 to 14 d was 10/m^2, and 4/m^2 from 15 to 28 d. The ambient temperature decreased with the age of the goslings (1 to 7d,

Table 4
Effect of different dietary treatments on growth performance of goslings from 1 d to 28d.

Items	CP (%)	S: P ratio	28 d weight (g)	ADG (g)	ADFI (g)	FCR
A	18	2.27 (Standard)	1763.52	59.97	116.96	1.95
B	16	2.70 (Standard)	1713.53	58.19	115.00	1.98
C	14	3.26 (Standard)	1739.86	59.12	120.72	2.04
D	18	2.06 (Reduced)	1795.85	61.13	118.50	1.94
E	16	2.47 (Reduced)	1797.30	61.18	123.70	2.02
F	14	2.97 (Reduced)	1762.35	59.93	120.51	2.01
SEM	8.074	0.167	9.497	0.010		
CP	18		1779.68	60.55	117.73	1.95^{a}
	16		1755.42	59.68	119.35	2.00^{b}
	14		1751.11	59.53	120.61	2.03^{b}
S: P ratio		Standard	1738.97^{a}	59.09^{a}	117.56^{a}	1.99
		Reduced	1785.17^{b}	60.74^{b}	120.90^{b}	1.99
P-value		CP	0.203	0.203	0.119	0.001
		S: P ratio	0.002	0.002	0.005	0.974
		Interaction	0.164	0.164	0.006	0.133

Note: [a,b]Means with different superscripts within the same row differ significantly ($P < 0.05$).

Table 5
Effect of different dietary treatments on serum biochemical indicators of goslings at 28 d.

Items	CP (%)	S: P ratio	TP (g/L)	ALB (g/L)	GLB (g/L)	GLU (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)	CREA-S (μmol/L)	UREA (mmol/L)
A	18	2.27 (Standard)	40.85	7.59	32.77	12.66	3.25	2.68	1.45	1.64	12.83	1.83
B	16	2.70 (Standard)	42.60	8.82	33.78	12.24	3.41	2.75	1.69	1.75	16.17	1.50
C	14	3.26 (Standard)	40.78	9.00	31.78	11.94	3.45	2.84	1.65	1.73	14.60	1.52
D	18	2.06 (Reduced)	40.00	8.75	31.58	11.39	3.39	2.56	1.82	1.85	14.50	1.65
E	16	2.47 (Reduced)	43.28	9.78	34.10	12.71	3.87	3.48	1.77	2.14	12.80	1.60
F	14	2.97 (Reduced)	42.90	9.75	33.15	10.52	3.74	3.08	1.70	1.90	11.67	1.80
SEM			0.265	0.063	0.670	0.218	0.492	0.167	0.039	0.047	0.418	0.060
CP	18		40.43	8.16^{b}	32.18	12.02	3.32^{b}	2.62	1.63	1.74	13.67	1.74
	16		42.94	9.30^{a}	33.94	12.48	3.64^{a}	3.12	1.73	1.94	14.48	1.55
	14		41.84	9.38^{a}	32.47	11.23	3.59^{a}	3.18	1.68	1.81	13.13	1.66
S: P ratio		Standard	41.41	8.47^{b}	32.78	12.28	3.37^{b}	2.76	1.60^{b}	1.70^{b}	14.53^{a}	1.62
		Reduced	42.06	9.43^{a}	32.94	11.54	3.67^{a}	3.19	1.76^{a}	1.96^{a}	12.99^{b}	1.68
P-value		CP	0.337	0.024	0.328	0.142	0.047	0.327	0.573	0.153	0.318	0.435
		S: P ratio	0.642	0.018	0.870	0.147	0.012	0.207	0.028	0.004	0.038	0.591
		Interaction	0.680	0.905	0.585	0.245	0.496	0.506	0.150	0.521	0.011	0.294

Note: [a,b]Means with different superscripts within the same row differ significantly ($P < 0.05$).

28−30°C 7 to 14 d, 26−28°C; 15 to 28 d, 24−26°C). Birds were provided with unlimited water and feed under natural light.

Data and sample collection, chemical analyses, calculations

Growth performance

All goslings were weighed at 1 d and 28 d to calculate WG and ADG. The feed intake of geese was counted weekly to calculate the average daily feed intake and F/G.

Serum biochemical indicators

5 mL of blood was collected from the brachial vein of goslings before slaughter and centrifuged at 3500 rmp for 10 min to produce serum samples. Serum samples were stored at -20°C for determination of serum biochemical indexes. Total protein (TP), albumin (ALB), globulin (GLB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG), high-density lipoprotein-c (HDL-c), low-density lipoprotein-c (LDL-c), creatinine (CREA), urea nitrogen (UREA) and glucose (GLU) were analyzed with the use of Hitachi 7600 Automatic Biochemistry Analyzer (Hitachi High-Tech Corporation, Tokyo, Japan).

Amino acid content

Serum and muscle amino acid content were measured according to GB 5009.124-2016 and Liang et al. (2023) method. Methionine content was determined by oxidative hydrolysis, and other free amino acids (alanine, glycine, glutamate, arginine, lysine, isoleucine, histidine, phenylalanine, tyrosine, Leucine, proline, serine, threonine, aspartate, valine) content were analyzed by acid hydrolysis.

Apparent starch digestibility

Starch digestibility in the intestine was analyzed by following Khoddami et al. (2017). In brief, at 28 day, after slaughtering, the abdominal cavities opened, and the small intestine was removed. The jejunum and ileum was separated and collected the chyme part from the distal jejunum and distal ileum with a 1.5 mL enzyme-free tube. The chyme was mixed, freeze-dried, grounded, and then weighed to measure the apparent starch digestibility coefficient-determination of starch concentration in feed and chyme by polarimetry. Titanium dioxide (TiO_2) was used as an inert marker, and the additional amount in the feed was 0.5%. The content of TiO_2 was determined by spectrophotometry, as described in Short et al. (1996). The apparent digestibility coefficient and disappearance rate of starch were calculated using the following formulas.

Table 6
Effect of different dietary treatments on serum animo acid content of goslings at 28 d. (nmol/L)

Items	CP (%)	S: P ratio	Ala	Gly	Glu	Arg	Lys	Ile	His	Phe	Met	Tyr	Leu	Pro	Ser	Thr	Asp	Val
A	18	2.27 (Standard)	20.02	16.72	38.51	12.06	22.00	9.66	6.02	10.90	14.74	9.57	22.75	15.78	19.81	15.81	26.92	15.79
B	16	2.70 (Standard)	21.14	16.65	40.10	12.63	23.37	10.17	6.24	11.50	14.97	9.94	23.25	16.50	20.91	15.53	28.05	16.65
C	14	3.26 (Standard)	19.08	14.92	37.79	11.71	22.02	9.61	5.85	10.54	15.14	9.27	21.50	15.46	19.01	16.11	26.00	15.36
D	18	2.06 (Reduced)	20.02	15.54	38.40	11.96	22.58	10.04	5.83	10.80	15.08	9.38	21.47	15.49	19.01	17.77	26.42	15.65
E	16	2.47 (Reduced)	19.75	15.62	37.65	11.94	21.62	9.76	5.75	10.41	15.28	9.16	21.35	15.28	18.63	17.29	25.85	15.36
F	14	2.97 (Reduced)	19.83	15.54	38.74	12.15	22.57	10.16	6.07	10.70	15.08	9.47	20.58	15.48	19.49	16.65	26.92	15.79
SEM			0.366	0.364	0.607	0.208	0.362	0.179	0.112	0.192	0.234	0.159	0.331	0.272	0.362	0.230	0.460	0.309
CP	18		20.02	16.13	38.46	12.01	22.29	9.85	5.93	10.85	14.91	9.47	22.11	15.64	19.41	16.79	26.67	15.72
	16		20.45	16.13	38.89	12.89	22.50	9.96	6.00	10.96	15.12	9.55	22.30	15.89	19.77	16.41	26.95	16.01
	14		19.46	15.23	38.26	11.93	22.30	9.89	5.96	10.62	15.11	9.37	21.04	15.47	19.25	16.38	26.46	15.58
S: P ratio		Standard	20.08	16.10	38.80	12.13	22.47	9.81	6.04	10.98	14.95	9.59	22.50^{a}	15.91	19.91	15.82^{b}	26.99	15.93
		Reduced	19.87	15.57	38.27	12.02	22.26	9.99	5.89	10.64	15.14	9.34	21.13^{b}	15.42	19.04	17.24^{a}	26.40	15.60
P-value		CP	0.583	0.542	0.926	0.795	0.970	0.970	0.969	0.781	0.926	0.913	0.240	0.838	0.842	0.639	0.920	0.865
		S: P ratio	0.784	0.489	0.680	0.796	0.782	0.646	0.527	0.396	0.705	0.455	0.039	0.395	0.248	0.001	0.543	0.615
		Interaction	0.528	0.565	0.561	0.596	0.374	0.559	0.493	0.422	0.937	0.503	0.818	0.663	0.335	0.295	0.437	0.568

Note: ^{a,b}Means with different superscripts within the same row differ significantly ($P< 0.05$).

Table 7
Effect of different dietary treatments on breast muscle animo acid content of goslings at 28 d. (%)

Items	CP (%)	S: P ratio	Ala	Gly	Glu	Arg	Lys	Ile	His	Phe	Met	Tyr	Leu	Pro	Ser	Thr	Asp	Val
A	18	2.27 (Standard)	0.80	0.96	2.02	0.94	1.10	0.62	0.31	0.67	0.21	0.40	1.03	0.54	0.54	0.59	1.20	0.62
B	16	2.70 (Standard)	0.77	0.85	2.02	0.91	1.10	0.61	0.32	0.66	0.22	0.39	1.01	0.51	0.55	0.58	1.15	0.61
C	14	3.26 (Standard)	0.66	0.90	2.09	0.90	1.06	0.58	0.32	0.66	0.22	0.40	0.98	0.56	0.51	0.54	1.14	0.62
D	18	2.06 (Reduced)	0.66	0.97	2.11	0.91	1.03	0.58	0.31	0.64	0.21	0.38	0.98	0.56	0.51	0.63	1.15	0.59
E	16	2.47 (Reduced)	0.70	0.94	2.06	0.90	1.02	0.58	0.31	0.62	0.22	0.37	0.97	0.55	0.51	0.61	1.19	0.58
F	14	2.97 (Reduced)	0.82	1.04	1.98	0.93	1.07	0.58	0.31	0.64	0.22	0.37	0.99	0.57	0.53	0.60	1.19	0.62
SEM	0.021	0.029	0.026	0.014	0.017	0.008	0.004	0.011	0.003	0.005	0.012	0.008	0.008	0.007	0.011	0.009		
CP	18		0.73	0.96	2.06	0.92	1.06	0.60	0.31	0.66	0.21	0.39	1.00	0.55	0.52	0.61^{a}	1.17	0.60
	16		0.73	0.90	2.04	0.90	1.06	0.59	0.31	0.64	0.22	0.38	0.99	0.53	0.53	0.59^{ab}	1.16	0.59
	14		0.74	0.97	2.03	0.91	1.06	0.58	0.31	0.65	0.22	0.39	0.99	0.57	0.52	0.57^{b}	1.17	0.62
S: P ratio		Standard	0.74	0.90	2.04	0.91	1.08	0.60	0.32	0.67	0.21	0.39	1.00	0.54	0.53	0.57^{b}	1.16	0.62
		Reduced	0.73	0.98	2.05	0.91	1.04	0.58	0.30	0.63	0.22	0.38	0.98	0.56	0.52	0.61^{a}	1.18	0.60
P-value		CP	0.974	0.562	0.905	0.806	0.996	0.655	0.876	0.858	0.750	0.824	0.877	0.146	0.981	0.021	0.933	0.464
		S: P ratio	0.719	0.198	0.901	0.985	0.202	0.231	0.126	0.135	0.824	0.094	0.379	0.117	0.271	0.001	0.583	0.321
		Interaction	0.011	0.649	0.293	0.730	0.592	0.575	0.987	0.926	0.935	0.952	0.577	0.610	0.254	0.648	0.137	0.872

Note: ^{a,b}Means with different superscripts within the same row differ significantly ($P< 0.05$).

Table 8
Effect of different dietary treatments on leg muscle animo acid content of goslings at 28 d. (%)

Items	CP (%)	S: P ratio	Ala	Gly	Glu	Arg	Lys	Ile	His	Phe	Met	Tyr	Leu	Pro	Ser	Thr	Asp	Val
A	18	2.27 (Standard)	1.16	0.90	2.81	1.28	1.73	1.02	0.63	1.00	0.30	0.62	1.65	0.69	0.75	0.89	1.80	1.06
B	16	2.70 (Standard)	1.05	0.85	2.44	1.25	1.68	0.94	0.60	0.89	0.30	0.56	1.57	0.59	0.73	0.81	1.72	0.96
C	14	3.26 (Standard)	1.13	0.86	2.70	1.24	1.68	0.98	0.60	0.97	0.28	0.57	1.60	0.63	0.75	0.89	1.75	1.00
D	18	2.06 (Reduced)	1.14	0.84	3.04	1.25	1.68	0.98	0.55	0.98	0.31	0.70	1.63	0.65	0.81	1.06	1.68	1.00
E	16	2.47 (Reduced)	1.09	0.85	2.83	1.22	1.63	0.95	0.62	0.96	0.29	0.64	1.57	0.62	0.75	0.91	1.74	0.99
F	14	2.97 (Reduced)	1.13	0.86	2.81	1.25	1.69	0.97	0.65	0.96	0.30	0.59	1.60	0.65	0.75	0.90	1.76	1.02
SEM	0.013	0.014	0.042	0.008	0.012	0.009	0.011	0.013	0.004	0.011	0.012	0.009	0.010	0.016	0.022	0.012		
CP	18		1.15[a]	0.87	2.93[a]	1.26	1.71	1.00	0.59	0.99	0.31	0.66[a]	1.64	0.67[a]	0.78	0.97[a]	1.74	1.03
	16		1.09[b]	0.85	2.64[b]	1.23	1.65	0.95	0.61	0.93	0.29	0.60[b]	1.57	0.61[b]	0.74	0.86[b]	1.73	0.97
	14		1.13[ab]	0.86	2.76[b]	1.25	1.68	0.97	0.62	0.96	0.29	0.58[b]	1.60	0.64[ab]	0.75	0.89[b]	1.76	1.01
S: P ratio		Standard	1.11	0.87	2.65[b]	1.25	1.70	0.98	0.61	0.95	0.29	0.58[b]	1.61	0.64	0.74	0.86[b]	1.76	1.00
		Reduced	1.12	0.85	2.89[a]	1.24	1.67	0.97	0.61	0.97	0.30	0.64[a]	1.60	0.64	0.77	0.95[a]	1.73	1.00
P-value		CP	0.028	0.865	0.003	0.261	0.192	0.069	0.438	0.107	0.164	<0.001	0.076	0.012	0.251	<0.001	0.887	0.182
		S: P ratio	0.715	0.596	0.001	0.322	0.185	0.509	0.825	0.496	0.237	<0.001	0.831	0.705	0.128	<0.001	0.498	0.820
		Interaction	0.638	0.652	0.216	0.510	0.481	0.411	0.032	0.281	0.205	0.218	0.968	0.119	0.402	0.010	0.409	0.308

Note: [a,b]Means with different superscripts within the same row differ significantly ($P < 0.05$).

Apparent starch digestibility = 100- $[(C_1 \times C_2)/ (C_3 \times C_4)] \times 100$

C_1: starch content in chyme;
C_2: dietary TiO_2 content;
C_3: dietary starch content;
C_4: TiO_2 content in chyme.

Enzyme activity

α-amylase and *maltase* activities of intestinal chyme were measured using kits (Nanjing Jincheng Bioengineering Institute, Nanjing, China). The two kits were mentioned as C016-1-1 and A082-3-1, respectively.

Relative gene mRNA expression

The kits for total RNA extraction, reverse transcription, and real-time PCR analysis were purchased from Yeasen Biotechnology (Shanghai) Co., Ltd. Briefly, total RNA was extracted from jejunal mucosal tissue according to the method provided by the manufacturer. RNA integrity was verified by determining RNA concentration and by 1% agar gel electrophoresis. Extracted RNA was diluted with sterile, enzyme-free water to maintain a consistent RNA concentration for each sample. Afterward, the total RNA from each sample was reverse-transcribed into cDNA using a reverse transcription system. The melt curve stage was programmed using the default settings of Applied Biosystems: 7500. The primer sequences used in this study are listed in Table 3. The β-action gene was used as the internal reference gene. All samples contained 3 biological replicates, and the results were analyzed using ΔCt values and the results were calculated by the $2^{-\Delta\Delta Ct}$ method and expressed as the mean value.

Statistical analysis

The results were statistically analyzed by one-way ANOVA (analysis of variance) and General Linear Model (GLM) using SPSS 26.0 (SPSS, Inc., Chicago, IL) software, and LSD was applied for multiple comparisons. The data was presented as the mean values and the standard error of the means (SEM). Differences were considered statistically significant at $P < 0.05$. The figures were created using Graph-Pad Prism 8 (Graph Pad Software Inc., San Diego, CA) software.

Results

Growth performance

The effect of reducing the S: P ratios at different protein levels on the growth performance of 28-day-old goosling is shown in Table 4. Lowering the protein level increased FCR at the same dietary S: P ratio type ($P < 0.05$), and also there was a trend toward increased ADFI ($P > 0.05$). Conversely, reducing the S: P ratio at the same dietary protein level led to a significant increase in body weight, WG, ADG, and ADFI of 28-day-old goslings ($P < 0.05$).

Serum biochemical indicators content

Serum biochemical indicators are shown in Table 5. Decreasing dietary protein levels and reducing the S: P ratio resulted in an increase ($P < 0.05$) in serum's ALB content. The ALB/GLB ratio was significantly higher ($P < 0.05$) at 14% than 18% of the protein level. However, a protein level of 18% resulted in a reduced serum TC content in 28-day-old goslings ($P < 0.05$). Reducing the dietary S: P ratio was associated with elevated serum TC, HDL-C, and LDL-C levels ($P < 0.05$), and decreased CREA-S levels ($P < 0.05$).

Serum amino acid concentration

Table 6 shows the effect of reducing the S: P ratios at different protein levels on serum amino acid concentration. Changes in dietary protein levels did not have a significant effect on the concentration of 16

Table 9
Effect of dietary treatments on the starch digestibility of distal jejunum & ileum and the activity of starch digestive enzymes in chyme of goslings at 28 d.

Items	CP (%)	S: P ratio	Distal jejunum			Distal ileum		
			Digestibility (%)	α-amylase (U/mg)	Maltase (U/mg)	Digestibility (%)	α-amylase (U/mg)	Maltase (U/mg)
A	18	2.27 (Standard)	84.85	198.38	462.11	93.02	370.29	696.19
B	16	2.70 (Standard)	86.86	189.16	468.03	92.45	382.57	588.12
C	14	3.26 (Standard)	86.11	201.66	473.18	92.84	314.96	618.24
D	18	2.06 (Reduced)	82.19	176.81	526.10	92.43	370.73	691.43
E	16	2.47 (Reduced)	78.55	153.86	472.43	93.61	406.01	663.68
F	14	2.97 (Reduced)	75.25	155.05	499.59	92.64	381.41	693.75
SEM	0.648	6.205	0.648	0.238	12.977	0.238		
CP	18		83.52	187.60	494.25	92.73	370.51	695.31
	16		82.80	171.51	470.23	93.03	394.29	625.90
	14		82.18	178.36	486.38	92.74	348.19	656.00
S: P ratio		Standard	85.94[a]	196.40[a]	467.87	92.77	355.94	634.18
		Reduced	79.66[b]	161.91[b]	499.37	92.90	386.05	683.95
P-value		CP	0.141	0.522	0.655	0.838	0.367	0.468
		S: P ratio	<0.001	0.005	0.155	0.790	0.259	0.283
		Interaction	<0.001	0.673	0.533	0.298	0.584	0.729

Note: [a,b]Means with different superscripts within the same row differ significantly ($P < 0.05$).

amino acids in serum ($P > 0.05$). However, differences were observed for Leucine and Threonine, whereas the concentrations of both amino acids increased in diets with reduced S: P ratios ($P < 0.05$).

Muscle amino Acid Content

Tables 7 and 8 shows the effect of reducing the S: P ratios at different protein levels on the amino acid content in breast and leg muscle. A reduction in dietary protein levels led to a decrease in Thr content in the breast muscle, as well as Ala, Glu, Tyr, Pro, and Thr content in the leg muscle ($P < 0.05$). Conversely, Thr content in breast muscle, and the Glu, Tyr, and Thr content in leg muscle increased in those group that fed reduced S: P ratio diets ($P < 0.05$).

Starch digestibility and starch-digesting enzyme activity

Outcomes related to starch digestibility and activity of starch-digesting enzyme in the distal jejunum and distal ileum are presented in Table 9. The interaction between protein levels and and S: P ration significantly increased starch digestibility ($P < 0.001$). A reduction in the S: P ratio resulted in a 7.31% decrease in starch digestibility in the jejunum ($P < 0.001$). Furthermore, lowering the S: P ratio diminished α-amylase activity in jejunal chyme ($P < 0.05$). Conversely, maltase activity showed a slight increase in both intestinal tracts when fed diets with reduced S: P ratios; however, these differences were not statistically significant ($P > 0.05$).

Relative gene mRNA expression

The relative mRNA expression of glucose and amino acid transporter genes is shown in Fig. 1. In groups with a reduced S: P ratio, the expression of the *SGLT1* and *GLUT2* genes was generally down-regulated, while the expression of the *SLC7A5* gene was up-regulated ($P < 0.05$).

Discussion

Generally, varied levels of dietary protein affects feed intake and FCR. High-protein diets reduce FCR by decreasing feed intake. In this experiment, when the dietary protein level was reduced from 18% to 14%, the FCR was elevated by 4.1%. Similar results were observed in geese diets with low-protein levels (Liang et al., 2023; Ho et al., 2015). However, it is not consistent. Abou-Kassem et al. (2019) research was inconsistent with the above; when the protein level was reduced from 22% to 18.05%, FCR was decreased from 2.91 to 2.58. Moreover, a previous study found no effect of different dietary protein levels on average daily feed intake and feed conversion ratio of fattening Turkey geese (Sahin et al., 2008). The all above researches revealed that feed protein levels have a variable effect on FCR in geese, and it is hypothesized that this might be related to breed, age, and feed formulation. In the following study the remarkable result is that, lowering the 10% S: P ratio of the diets at the same protein level improved body weight gain. The reason may be the lower digestibility and slower disappearance rate of starch in the jejunum, resulting in more starch entering the ileum to be hydrolyzed into glucose, followed by glucose breakdown for intestinal energy supply (Selle et al., 2019). The conclusions of the two experiments by Greenhalgh et al. (2020, 2022) are restrictive; that is, the dietary protein level should be reasonable. When protein levels were too low, broilers showed poor growth performance, whether fed on a standard or a reduced S: P ratio diet. However, in the present experiment, even if the protein was reduced to 14%, the growth performance of goslings was not affected, this might be attributed to the supplementation of amino acids. Moreover, one difference between geese and broilers is that goose is herbivorous, and its response to protein concentration is less sensitive than that of broiler. This aspect may explain why lowering dietary protein levels did not affect body weight gain in this experiment.

When the diet's corn (starch) content was reduced, soybean oil was preferred to provide energy to keep every group's energy levels consistent. It should be known that the energy density of oil is much higher than corn, and its energy supply efficiency is higher than that of starch, which might be helpful for geese to achieve better growth performance (Palmquist et al., 1980). However, starch, as the primary energy source, cannot be replaced by fat. Excessive oil in the ration may decrease the feed intake of geese (Martinez et al., 1995) and slowdowns the emptying procedure of the chyme in intestine. To minimize the effect of soybean oil in the experiment, it is necessary to control the starch-to-lipid ratio (Khoddami., 2018). Therefore, the amount of soybean oil added to all reduced diets did not exceed 2%, and the changes in blood lipid indexes might be associated to additional soybean oil.

As feed moves through the digestive system, it subjected to various physical and chemical processes. After feeding on starch, it initially digested by oral salivary amylase, softened by crop mucus, mixed by the glandular stomach in the crop, and grounded by muscles. Then, it enters the small intestine in close contact with digestive fluid. Through the action of α-amylase, straight-chain starches hydrolyzed into maltose and maltotriose, and some branched-chain starches decomposed into maltose, maltotriose, and α-dextrin (Wiseman, 2006). Other branched-chain starches require further degradation of the α-1,6 glycosidic bond of α-dextrin by isomaltase released from the brush

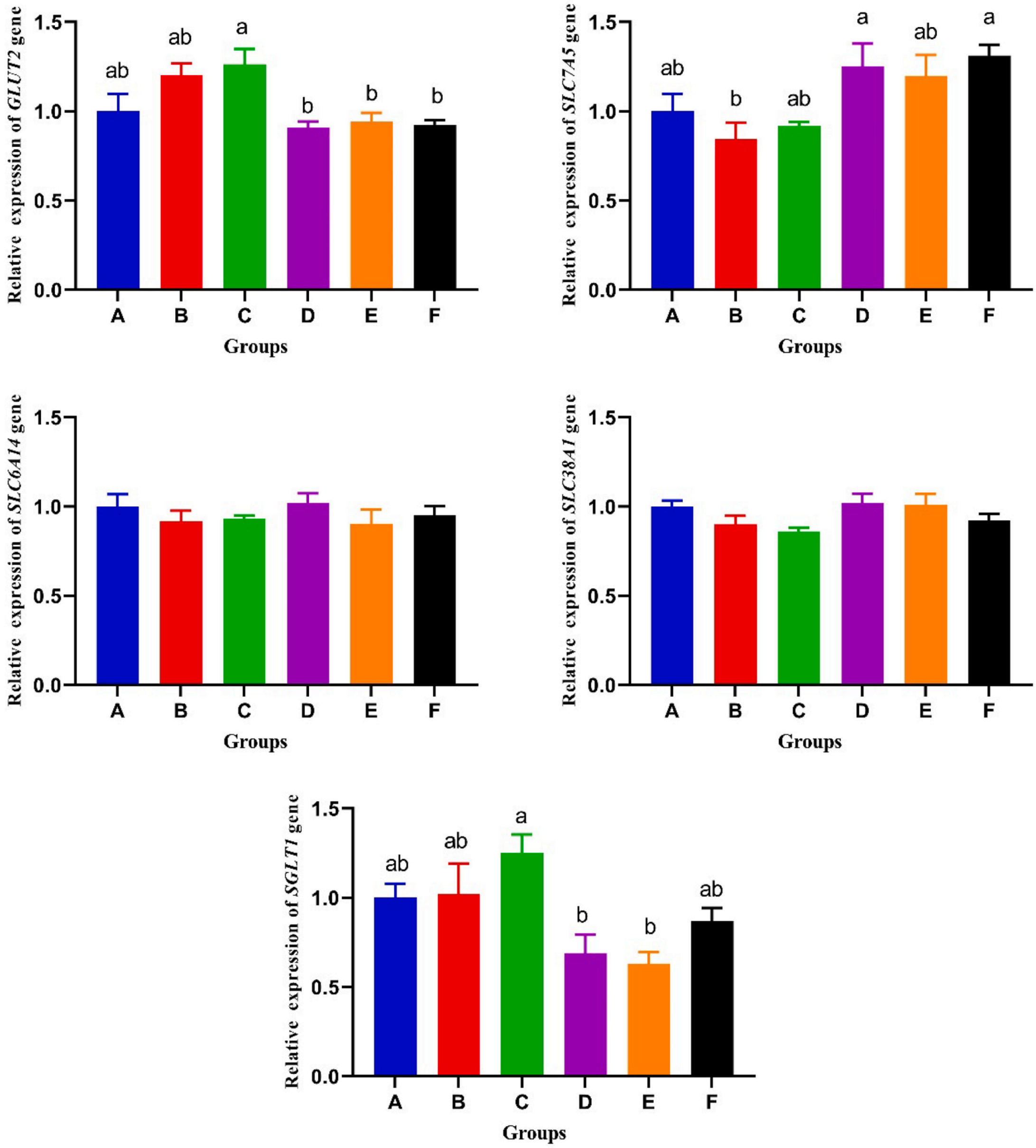

Fig. 1. Relative gene expression of glucose and amino acid transport carriers
Note: A: High protein, Standard S:P ratio; B: Medium protein, Standard S:P ratio; C: Low protein, Standard S:P ratio;D: High protein, ReducedS:P ratio; E: Medium protein, Reduced S:P ratio; F: Low protein, Reduced S:P ratio. [a, b]Means with different superscripts within the same row differ significantly ($P< 0.05$).

border membrane on the surface of the small intestine (Tester et al., 2003). These disaccharides hydrolyzed ultimately into monosaccharides by oligosaccharides and eventually converted into glucose, which is absorbed by the intestinal wall into the bloodstream and participates in body metabolism (Svihus, 2014; Nichols et al., 2003). The primary site of starch digestion in geese is the small intestine. It was reported that about 65% of the starch is digested till it reaches the end of the duodenum, 85% at the end of the jejunum, and 97% at the end of the ileum (Riesenfeld et al., 1980). Outcomes presented that reducing the S: P ratios in diets resulted in a 17.56% attenuation of α-amylase activity in the jejunum, a trend consistent with changes in starch digestibility. Amylase activity positively correlates with the amount of starch consumed (Huntingtin, 1997). The diminished starch content of the diet made it less necessary for the digestive tract to secrete as much amylase to digest the starch.

In the early growth of Jiangnan White Goose, the development of leg muscles is faster than that of breast muscles. Therefore, more amino acids will be transported from the liver to the leg muscles through the blood to synthesize protein. If the blood amino acids change, the amino acid composition of the leg muscles will be affected more than that of the breast muscles. Gene expression of the glucose transporter vectors *SGLT1* and *GLUT2* was down-regulated, whereas gene expression of the amino acid transporter vector *SCL7A5* was up-regulated in the reduced S: P diets. In short, we speculate that there was a competitive relationship between glucose and amino acids, and more amino acids enter the portal vein, which may elucidate why the content of some amino acids in muscles, especially in leg muscles, was increased.

In addition, some branched-chain amino acids such as leucine and threonine will affect the growth rate of intestinal cells and the activity of digestive enzymes. When the absorption of intestinal amino acids is affected, the activity of starch-digest enzymes will also change (Cao et al., 2018; Yu et al., 2013). On the one hand, leucine and threonine, as substrates of protein synthesis, regulate the expression of relevant functional proteins and the synthesis of functional enzymes in the digestive system, and therefore, affect the growth rate of intestinal cells and digestive enzyme activity (Yang et al., 2019; Zhang et al., 2017). On the other hand, as energy donors for intestinal epithelial cells, leucine, and threonine can affect the proliferation of intestinal epithelial cells (Guo et al., 2018).

Conclusion

Reducing the starch: protein ratio of the diet has been shown to improve growth performance of goslings. This improvement might be attributed to slower starch digestibility, which allows more amino acids pass through the intestinal mucosa due to competition with glucose. This factor is significant and should not be overlooked. Based on this study, it is recommended to use a diet with an energy density of 11.5 MJ/kg, a protein level of 14%, and a starch: protein ratio of 2.97 for goslings from 1 to 28 days of age.

Declaration of AI and AI-assisted technologies in the writing process

During the preparation of this work the author used *Grammarly* in order to improve readability and language. After using *Grammarly*, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Disclosures

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Zhiyue Wang reports financial support was provided by CARS-42-11. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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