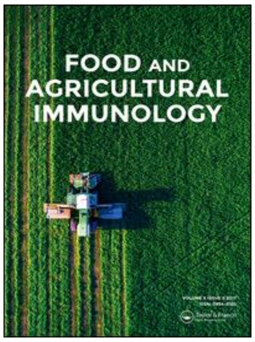

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Effects of dietary supplementation of wheat starch processing wastewater on growth performance, nutrient digestibility and immune function in sheep

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ABSTRACT
The objective was to investigate the effects of dietary supplementation of wheat starch processing wastewater (WSW) on growth performance, nutrient digestibility and immune function in sheep. Eighty male Hu sheep were divided randomly into five treatments. Five groups of sheep were fed isocaloric and isonitrogenous diets containing 0 (M1), 0.5% (M2), 1% (M3), 1.5% (M4) and 2% (M5) WSW, on dry matter (DM) basis, respectively. We found that the disappearance for the DM, crude protein (CP), gross energy(GE), ash and organic matter of WSW at 48 h were 62.52%, 64.32%, 65.62%, 55.78%, 62.74%, respectively. The feed: gain (F:G) for M5 was 0.77% and 0.64% less than M1and M3, respectively ($P < .05$). Serum characteristics (IgA, IgG, IgM and total protein) were not affected by WSW. These results indicated that fed diets containing up to 2% DM of WSW had reduced F:G and increased CP digestibility.

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Wheat starch wastewater; growth performance; digestibility; immune function; sheep

Introduction

By-products of wheat have long been employed as ingredients in animal feeds. Processing wheat starch can produce large amounts of wastewater (also called pentosan phase). About 5~12 t wheat starch wastewater (WSW) will be required to produce each ton wheat starch (Ma et al., 2006). WSW contains high concentrations of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), resulting in its short shelf-life. If not recycled or processed quickly, the wastewater will cause serious resource waste and environmental pollution. Some companies obtain vacuum-concentrated products by spray and drying technology. The dry matter of WSW is low (4%), and it costs much to concentrate it, so some factories fermented it to produce yeast (Yang et al., 2013), alcohol or monosodium glutamate (Zhou, Liu, & Lv, 2003), which would produce second pollution.

Non-starch polysaccharides systems (NSPs) have a structural function as the main component of plant cell walls, and account for approximately 10% of the whole grain

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in wheat. The majority of NSPs in wheat are pentosans, which account for about 6% of the grain by dry weight. Pentosan also has similar function as dietary fibre, such as maintaining blood glucose, reducing blood cholesterol levels and other physiological functions. It also increased faecal short chain fat acid indicating that pentosan may have favourable effects on colonic health and potential in improving gut function. In addition, pentosan can be enzymatically hydrolysed by xylanase to produce xylooligosaccharide (XOS) (Bocchini, Oliveira, Gomes, & Silva, 2005). And some fungi and bacteria, which were abundant in rumen from ruminants, could produce xylanase through fermentation. Some results suggest that XOS may be beneficial in stimulating intestinal bifidobacterium without having much effect on *Lactobacillus* (Li, Summanen, Komoriya, & Finegold, 2015). *Lactobacillus plantarum* produced organic acids which combined with metallic ions to synergistically improve the antioxidant capacity (Wang et al., 2017). GSH-Px levels were lowest in the low-density group and highest in the medium-density group (Li et al., 2017). Some kinds of processing waste water have been utilized as feed stuff for ruminant, such as corn processing wastewater, corn steep liquor, due to their relatively high energy and crude protein. However, there are seldom researches on the direct feeding usage for WSW in ruminant feeds. The aim of the present study was to investigate the effects of different levels of WSW on growth performance, apparent digestibility and immune functions in sheep.

Materials and methods

Experiment 1: the digestibility of wheat starch wastewater on sheep rumen

Preparation of wheat starch wastewater powder

The WSW was provided by ZhongHe Group and dried at 65 °C in an oven. The contents of DM, OM, CP, GE, Ash, starch and pentosan were detected.

Animals and diets

Degradability of WSW powder was measured by the "in vitro gas production" method. DM, Ash, OM, CP and GE degradability were determined in three Hu sheep. The animals (weighing 30 ± 1 kg) fitted with cannula in the rumen were kept in individual cages and fed a standard diet (12 MJ/Kg DE, 13% CP) consisting of 50% concentrate (containing 42% corn, 27% bran, 11.5% soybean meal, 12% cottonseed meal, 0.5% limestone, 1%CaHPO4, 1.5% NaCl, 2% premix, 2.5% $NaHCO_3$), 10% peanut vines and 40% whole corn silage. Sheep were fed (total amount of dry matter was 1 kg) two times a day (7:00 and 16:00) and had free access to water.

In situ *study*

Rumen fluid was collected at 6:00 in the morning, and an equal volume of fluid from the three sheep was pooled and mixed in a thermos flask pre-warmed to 39°C. The fluid was then mixed with an artificial rumen gastric juice (1:2, V/V) with a mineral solution containing 8.75 g $NaHCO_3$, 1 g NH_4CO_3, 1.55 g KH_2PO_4, 0.15 g $MgSO_4{\cdot}7H_2O$, 0.52 g Na_2S, 0.017 g $CaCl_2{\cdot}2H_2O$, 0.015 g $MnCl_2{\cdot}4H_2O$, 0.002 g $CoCl_3{\cdot}6H_2O$, 0.012 g $FeCl_3{\cdot}6H_2O$ and 0.125 g reazurin per litre. Then it was filtered and flushed with CO_2. Gas production (GP) was read immediately, and then read at 2, 6, 12, 24, 36 and 48 h of incubation.

To describe the dynamics of *in vitro* GP over time, Gompertz function was used as

$$GP = A \times exp\left\{-\exp\left[1 + \frac{b \times e}{A}(LAG - t)\right]\right\},$$

where GP is the volume of GP (ml) at time t, b is the maximum rate of GP (ml/h), A is the maximum GP corresponding to complete substrate digestion and LAG is the lag time (h) defined as the time-axis intercept of a tangent line at the point of inflection. The constant e is 2.718. The parameters A, b and LAG were estimated by the nonlinear regression analysis procedure of SAS software (SAS Institute Inc., NC, USA).

Experiment 2: effect of dietary wheat starch wastewater supplementation on growth performance and immune function in sheep

Animals and management

After 7 days of adaption periods for diets, 80 male Hu sheep (16.18 ± 0.5 kg of body weight) were divided randomly into 5 treatments ($n = 4$, 4 sheep/pen) for 97 days of trial. Five groups of sheep were fed isocaloric and isonitrogenous diets containing 0 (M1), 0.5% (M2), 1% (M3), 1.5% (M4) and 2% (M5) WSW (DM basis). The basal diet (Table 1) was formulated according to Agricultural Research Council (NRC) requirement for sheep (1985). All the sheep were given the same feeding amount daily, according to the formula of $Y = 0.0147 \times BW^{0.6176}$ ($R^2 = 0.9998$) (according to a preliminary experiment, Y indicated daily feed intake). The dry matter content of the diet was 26%, 38.5%, 51%, 63.5% and 76% respectively. All the sheep were provided ad libitum access to fresh

Table 1. Ingredients and nutrient composition of diets fed to sheep.

	Compositions				
Items	M1	M2	M3	M4	M5
Ingredient (% of DM)					
Whole corn silage	39.6	39.60	39.60	39.60	39.60
Peanut vine	30	30.00	30.00	30.00	30.00
Sodium bicarbonate	0.4	0.40	0.40	0.40	0.40
Corn bran	1.70	1.50	1.10	1.00	1.00
Bran	4.00	3.70	2.60	1.50	1.00
Wheat middling	7.50	7.50	7.60	7.30	6.70
Corn gluten meal	6.00	6.00	6.10	6.20	6.30
Corn germ meal	6.00	6.00	6.00	6.00	6.00
Sugar residue	2.80	2.80	3.60	4.50	5.00
Limestone	0.10	0.10	0.10	0.10	0.10
$CaHPO_4$	0.30	0.30	0.30	0.30	0.30
NaCl	0.60	0.60	0.60	0.60	0.60
Premix[a]	1.00	1.00	1.00	1.00	1.00
Wheat starch wastewater powder	0	0.50	1.00	1.50	2.00
Nutrient composition					
DM(%)	100.00	100.00	100.00	100.00	100.00
ME (MJ/kg)	10.05	10.05	10.03	10.02	10.00
CP (%)	13.50	13.53	13.53	13.54	13.53
ADF (%)	27.64	27.77	27.61	27.49	27.40
NDF (%)	50.21	49.51	48.91	48.52	48.27
Ca (%)	0.72	0.73	0.73	0.73	0.73
P (%)	0.36	0.35	0.34	0.34	0.33

[a]Contained the following per kg of premix:25 g saccharomycetes, 25 g cysteamine, 119 g $MgSO_4 \cdot 7H_2O$, 2.5 g $FeSO_4 \cdot 7H_2O$, 0.8 g $CuSO_4 \cdot 5H_2O$, 3 g $MnSO_4 \cdot H_2O$, 5 g $ZnSO_4 \cdot H_2O$, 10 mg Na_2SeO_3, 40 mg KI, 30 mg $CoCl_2 \cdot 6H_2O$, 95,000 IU vitamin A, 17,500 IU vitamin D and 18,000 IU vitamin E.

water, and fed twice daily at 07:00 and 17:00 h. Sheep were dewormed with lvermectin before commencement of the experiment. All the sheep were weighed at the beginning and the end of the experiment.

Sampling procedure

Diet offered and orts were recorded daily. Diet, orts and faeces samples were collected and pooled for 3 days to create composite samples for each repetition in the middle and at the end of the feeding period, respectively. One hundred grams of daily faecal matter without impurity was mixed with 0.5 ml 10% sulphuric acid. All the samples were stored at −20°C. When the experiment began, diet, orts and faecal samples were dried at 65°C for 48 h. Dried samples were ground through a 1-mm screen for apparent digestibility determination using the method of endogenous tracer (acid insoluble ash).

At the 42 days and 91 days of the experiment, 10 ml blood samples were collected from the jugular vein from two sheep in each replicate at 06:00 h. All the blood was collected into coagulant-treated tubes (Eppendorf, Hamburg, Germany) to obtain blood serum, thereafter centrifuged at $3000 \times g$ for 15 min at 4°C. Then, samples were stored at −20° C to analyse immunoglobulin IgG, IgM, IgA and TP.

Chemical analysis

Nitrogen was determined(AOAC, 1990)using Macro-Kjeldahl N analyser (FOSS, Denmark). Acid detergent fibre (ADF) and Neutral detergent fibre (NDF) were determined according to the method of Van Soest, Robertson, and Lewis (1991) using a fibre analyser (ANKOM Technology, New York, USA). Ash and ether extract (EE) use the method of standard (AOAC, 1990). DM, ash, OM and GE degradability were estimated using the equation according to Orskov and McDonald (1979). The IgM, IgG, IgA and TP concentrations in serum were analysed using commercial kits of Jiancheng Biology Co., Nanjing, China. Pentosan was determined according to the method of Douglas. Starch was determined using the method of enzymatic hydrolysis.

Statistical analysis

Statistical analyses were performed by one-way ANOVA using the SAS Statistical Software. Differences among treatment means were determined using the Student–Newman–Keuls multiple comparison test. A P-value $\leq .05$ was considered statistically significant.

Results and discussion

The digestibility of wheat starch wastewater

In the United States, approximately 14 million tons of corn steep liquor (a main by-product of corn starch processing), roughly 25–30%, are used as feed products. The development of the corn refining industry has made increasing amounts of great quality animal feedstuff ingredients available that exported to the United States and world markets. Compared with dry diet, using liquid by-products could decrease the negative effects on the environment and feeding costs by 10 to 17%. In our experiment, the largest average consumption of wastewater was 2 kg daily for each sheep. In this study, all of the feed stuff were corn and wheat by-products, without soybean products and only a little corn grain in the whole corn silage. The results showed that there were no adverse effects on growth in all the sheep, which was similar to the results of Hall (Hall & Chase, 2014). So, it is available to replace corn grain and soybean products by wheat and corn by-products in sheep feed.

The disappearance of DM and CP increased with increasing time of incubation. The contents of DM (42 g/kg fresh weight), CP (51.8 g/kg DM) and pentosan (254.39 g/kg DM) in our wastewater were lower than others (Haska, Nyman, & Andersson, 2010; Yan, Xiang, & Yang, 2014; Zhou et al., 2003). The content of starch (473.68 g/kg DM) was higher than Zhou, but lower than Haska. That was mainly because of their different processing technology for wheat.

Haska showed that waste streams from the processing of wheat flour contained significant amounts of indigestible carbohydrates, fructan of about 47 g/kg, xylose, 38 g/kg, arabinosyl, 58 g/kg, arabinogalactan, 14 g/kg and so on (Haska et al., 2010). Wheat-bran fractions contained relatively high levels of pentosans of about 27.5%, compared with pentosan content in wheat (nearly about 2%). It has been confirmed by some experts that pentosans in the food are to a large extent digested during passage through the rumen of sheep. Rumen microbial activity enables to make use of pentosans. Two species of obligate anaerobic pentosan-fermenting bacteria have been found in the rumen, Gram-negative, curved rods. The wheat-flour pentosan hydrolysats contained arabinose, xylose and a barely detectable trace of xylobiose. Through hydrolysed pentosan partially fractionated on a charcoal column, yielding arabinose and xylose, xylobiose, xylotriose, xylotetraose and xylopentaose, together with a series of high oligosaccharides. B Ling implied that dietary addition of Isomalto oligosaccharide could improve the rumen fermentation of growing sheep to some degree (Ling, Qu, Lu, & Liu, 2007). XOS have been shown to maintain gastrointestinal health and improve biological availability of calcium. However, many soluble fibres, both oligo- and polysaccharides, are relatively fast fermented and can cause bloating and discomfort when consumed in even amounts. In this experiment, the amount of pentosan, oligo- and polysaccharides was too low to cause discomfort. Since pentosan could be rapidly hydrolysed by rumen bacteria and produce oligosaccharides at the same time, the WSW could play a role of certain health care function. The wheat starch wastewater contained about 473.68 g/kg starch. The rumen is the major site of starch digestion in sheep. The main carbohydrate sources of the ruminants usually contain barley, maize and wheat, because they are cost-effective sources of digestible energy, while starch is the major nutrient providing energy. The wheat- or maize- based diets would had high digestibility of DM, OM, CP and EE than barley- or other based diets (Mosavi, Fatahnia, Alamouti, Mehrabi, & Kohi, 2011). The ruminal degradation of wheat starch was 32%/h (Wang et al., 2009). It is generally recognized that the most efficient utilization of energy from starch is when it is digested in the small intestine and absorbed as glucose rather than when starch is fermented in the rumen and converted fractional from propionate to glucose in the liver (Reynolds, 2006). Because wastewater mainly contains pentosan and starch, we can infer that it has high digestibility. The DM, OM, CP, GE digestibility of the wastewater powder was 62.52%, 62.74%, 64.32%, 65.62% at 48 h (Table 2), respectively, which just proved our inference was correct and some WSW would be digested in the small intestine (Tables 3 and 4).

Table 2. Chemical composition of wheat starch wastewater (g/kg dry matter).

Items	Wheat starch wastewater
Dry matter (g/kg fresh weight)	42
Organic matter	988.77
Crude protein	51.80
Starch	473.68
Pentosan	254.39
Gross energy(MJ/kg)	14.65
Ash	11.23

Growth performance

The initial body weight, finished body weight and average daily gain (ADG) had no significant difference ($P > .05$) (Table 5). The M5 group had the highest ADG. The maximum total consumption of wheat starch wastewater for 16 sheep of M5 was 164.01 kg/day, and the average consumption was 2.00 kg/day. The DM intake of the five feeding treatment groups did not differ ($P > .05$). But the F:G for M5 was, respectively, 0.77% and 0.64% less than M1 and M3 ($P < .05$). In our experiment, only M1 died one sheep may be because of vaccination, the others lived very well. Due to the high viscosity contributing to their negative effects, the wheat NSPs have been well documented to have anti-nutritive properties. Therefore, diets are often treated with NSP-degrading enzymes, reducing viscosity and improving nutrient availability and growth performances of broilers (Olukosi, Cowieson, & Adeola, 2007). Pirgozliev and Rose indicated a high pentosan content could reduce chickens daily feed intake and weight gain, but feed efficiency was not affected by pentosan content, and adding exogenous xylanase did not influence the growth performance of the birds compared to the non-pentosan group (Pirgozliev et al., 2015). Yin also reported that supplementing WSW and enzyme had no significant differences in ADG, average feed gain, F/G, diarrhoea rate and faeces index in piglets (Yin et al., 2016). In our experiment, there were no significant differences for ADG and average daily feed intake (ADFI) among treatments, but F/G of sheep in the M5, M4, M2 groups were significantly lower than those in M1 and M3 groups ($P = .0318$). Garcia also indicated wheat by-product did not affect ADFI and ADG but increased feed efficiency in weaned pig diets (Garcia et al., 2015).

The digestibility of nutrients in the experimental diets

Increasing the dietary level of wastewater increased DM and ash digestibility, extremely increased CP digestibility ($P < .01$), but had no effect on GE, fat, NDF and ADF digestibility at the 7th week. At the 13th week, the increasing wastewater had no effect on the DM, ash, GE, CP, fat, NDF and ADF digestibility. The effect of WSW on diet digestibility might be mainly affected by the total amount of WSW in the diet and by the composition of the diet. With the increasing level of WSW, the moisture content of the diet increased. The high level of moisture content probably decreased the feed intake, but increased the digestibility of the diet. Jensen and Mikkelsen summarized the results of 9 in vivo trials comparing the performance of pigs fed dry feed and liquid feed; liquid feed could improve weight gain by 4.4% and feed efficiency by 6.9% (Jensen, Mikkelsen, Garnsworthy, & Wiseman, 1998). Plumed-Ferrer and Niba et al. proved that the most common slurry given to pigs involves a feed to water ratio between 1:2 and 1:3 (Niba, Beal, Kudi, & Brooks, 2009;

Table 3. Wheat starch wastewater powder-disappearance of DM, CP, GE, ash and OM in incubation intervals of 2, 6, 12, 24, 36 and 48 h in sheep ($n = 3$).

	Time of incubation(hours)											
	2		6		12		24		36		48	
Disappearance	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
DM	31.90	10.01	46.24	16.10	47.86	9.13	53.61	16.21	64.98	9.12	62.52	1.13
CP	28.62	2.45	41.91	12.16	43.96	5.00	56.30	8.21	63.02	10.25	64.32	4.55
GE	24.61	15.31	38.91	18.13	45.83	19.00	59.39	7.13	71.40	2.23	65.62	2.11
Ash	28.03	5.12	39.68	16.14	38.50	6.00	48.87	15.12	46.97	10.21	55.78	11.31
OM	32.09	10.14	49.69	15.22	48.14	9.10	63.72	8.31	71.95	3.51	62.74	1.15

Table 4. *In vitro* GP of wheat starch wastewater powder.

	Wheat starch wastewater powder ERD									
	DM		Ash		OM		CP		GE	
Items	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Maximum GP (ml/200 mg DM)	0.72	0.01	0.58	0.08	0.71	0.00	0.67	0.03	0.73	0.06
GP rate (ml/h)	0.03	0.02	0.01	0.01	0.02	0.00	0.02	0.01	0.03	0.02
Lag time(h)	−15.79	7.83	−20.10	6.34	−12.09	3.22	−13.86	6.70	−6.11	5.46

Table 5. Effects of dietary supplementation with wheat starch wastewater on growth performance in sheep.

	Treatment					*P*-value
Items	M1	M2	M3	M4	M5	
Total consumption of wastewater for each sheep (kg)	0	41.00	82.01	123.01	164.01	
Average consumption of wastewater for each sheep (kg/day/sheep)	0	0.50	1.00	1.50	2.00	
Initial body weight (kg)	15.93 ± 2.77	16.21 ± 2.14	15.96 ± 2.19	16.37 ± 2.24	16.55 ± 2.19	.9370
Finished body weight (kg)	30.83 ± 4.54	35.36 ± 5.54	34.42 ± 3.94	35.31 ± 3.94	36.19 ± 3.30	.8318
ADG (g/day)	204 ± 33	208 ± 44	200 ± 30	205 ± 37	213 ± 32	.8919
ADFI (kg)	0.88 ± 0.07	0.83 ± 0.08	0.87 ± 0.07	0.83 ± 0.06	0.80 ± 0.10	.0552
Feed efficiency (g/g)	4.42 ± 0.28^{a}	$3.81 \pm 0.42^{b,c}$	$4.29 \pm 0.33^{a,b}$	$3.88 \pm 0.25^{b,c}$	3.65 ± 0.56^{c}	.0318
Liveability, %	93.75	100.00	100.00	100.00	100.00	

Notes: $^{a-c}$Within a given row, small letter superscripts indicate a significant difference ($P < .05$) between the values. Identical small letter superscripts indicate no significant difference ($P > .05$).

Plumed-Ferrer & Wright, 2009). Delivering diets in a liquid form facilitates hydration of the substrate before entry into the intestine (Blaabjerg, Jorgensen, Tauson, & Poulsen, 2011) and promotes contact with the rumen microbes. In liquid diets, much, if not all, of the soluble carbohydrate can be converted by microbial fermentation to volatile fatty acids (VFAs) and alcohol before consumption. Thus, the DM digestibility of M5, M4, M3 were significantly higher than M1 and M2. To make the isonitrogenous diets, increasingly corn gluten meal was added to M3, M4 and M5. Corn cluten meal was a high quality of protein feed material, may cause the digestion of CP significantly higher than M1 and M2. Danicke indicates the digestibility of crude protein at the terminal ileum decreased with the increasing of dietary concentration, but xylanase supplementation improved praecaecal crude protein digestibility significantly, because the xylanase addition increased generally the digestibility of amino acids at the terminal ileum (Danicke et al., 1999). In this experiment, xylanase was not available, but the bacteria in the rumen could digest the NSPs, which may have the same function as xylanase, and may lead to the improvement of the CP digestibility (Table 6).

Immune parameters

The physiological effects of wheat bran may provide the prevention of diseases such as colon and breast cancers, cardiovascular disease, obesity and gastrointestinal diseases (Stevenson, Phillips, O'sullivan, & Walton, 2012). Whole wheat has important implications for health, by acting as modulator of immune function and redox status (Molinari et al., 2009). Chitosan pentamer and chitosan hexamer in vivo could promote gene transcription and protein

Table 6. The digestibility of nutrients in the experimental diets.

	Treatments					
Apparent digestibility	M1	M2	M3	M4	M5	*P*-value
The 7th week						
Dry matter	63.14 ± 5.40[b]	66.03 ± 5.68[b]	69.52 ± 2.54[a]	68.88 ± 3.19[a]	72.94 ± 3.22[a]	.0476
Ash	64.09 ± 4.51[a]	61.22 ± 3.66[a,b]	55.80 ± 1.70[b]	58.05 ± 5.27[b]	65.99 ± 2.87[a]	.0105
Gross energy	66.95 ± 4.90	68.86 ± 5.96	72.07 ± 1.93	72.65 ± 2.24	73.74 ± 3.99	.1598
Crude protein	62.48 ± 1.63[b,c]	58.54 ± 6.69[c]	68.52 ± 4.06[a,b]	70.20 ± 2.30[a]	73.19 ± 5.79[a]	.0023
Fat	65.77 ± 2.30	66.85 ± 0.85	63.08 ± 1.56	63.08 ± 1.56	65.02 ± 2.06	.0712
NDF	64.83 ± 7.25	69.09 ± 3.75	64.90 ± 2.62	63.08 ± 3.53	71.44 ± 1.57	.0701
ADF	67.51 ± 5.74	69.93 ± 3.73	64.22 ± 2.72	64.17 ± 2.33	69.62 ± 5.51	.1922
The 13th week						
DM	62.99 ± 3.99	59.42 ± 1.99	59.57 ± 1.49	63.92 ± 7.23	66.84 ± 3.70	.1157
Ash	60.75 ± 1.98	62.85 ± 1.95	63.93 ± 2.03	62.99 ± 2.33	64.09 ± 4.90	.5086
Energy	68.30 ± 1.67	66.65 ± 3.77	68.59 ± 1.36	70.86 ± 6.85	73.13 ± 2.27	.1827
Protein	61.45 ± 0.06[b]	58.75 ± 3.02	65.74 ± 4.01	60.49 ± 8.62	66.40 ± 2.87	.2343
Fat	64.14 ± 4.74	66.96 ± 4.16	68.73 ± 2.38	70.51 ± 1.19	68.01 ± 3.23	.1556
NDF	62.22 ± 3.83	63.67 ± 5.39	62.27 ± 1.96	64.12 ± 4.05	69.07 ± 0.17	.0891
ADF	62.54 ± 3.02	63.38 ± 3.95	64.15 ± 1.48	63.61 ± 1.95	66.36 ± 3.52	.4594

Notes: [a–c]Within a given row, small letter superscripts indicate a significant difference ($P < 0.05$) between the values. Identical small letter superscripts indicate no significant difference ($P > .05$).

Table 7. Serum IgG, IgA, IgM and TP levels(g/l) at the 7th and 13th weeks.

	Treatment					
Items (g/l)	M1	M2	M3	M4	M5	*P*
The 7th week						
IgG	5.14 ± 1.35	5.38 ± 1.06	5.20 ± 0.93	6.04 ± 1.00	5.17 ± 0.72	.4397
IgA	2.61 ± 0.59	2.29 ± 0.28	2.80 ± 0.97	2.97 ± 0.79	2.64 ± 0.88	.4576
IgM	4.12 ± 1.01	4.31 ± 1.67	4.15 ± 0.97	5.16 ± 1.29	4.50 ± 1.43	.3913
TP	5.31 ± 0.78	5.73 ± 1.03	5.80 ± 0.89	5.73 ± 0.49	5.92 ± 1.07	.7421
The 13th week						
IgG	6.22 ± 1.55	6.45 ± 1.32	5.91 ± 1.11	5.48 ± 1.14	6.57 ± 1.45	.5422
IgA	2.53 ± 0.82	1.82 ± 0.55	2.39 ± 1.01	2.56 ± 1.74	2.50 ± 1.08	.6555
IgM	6.47 ± 0.65	6.00 ± 0.61	5.82 ± 0.65	6.44 ± 0.65	6.46 ± 0.65	.9255
TP	5.95 ± 1.25	5.43 ± 0.91	5.79 ± 0.77	5.53 ± 0.76	6.06 ± 1.52	.8064

secretion of cytokines; thus the immuno-modulating ability was promoted (Wei, Wang, Zhu, Xiao, & Xia, 2009). While the by-products of wheat gluten can improve the growth performance, serum superoxide dismutase and intestinal antioxidant activities of the broiler chickens (Fang et al., 2017). Bailly reported adding XOS in the diet could improve the bile IgG by 14.2% (Bailly et al., 2001). At the 7th and 13th weeks, the blood serum characteristics IgM, IgG, IgA and TP were not affected by the WSW (Table 7). Many experiments have indicated that oligosaccharides could enhance immunity (Gao & Shan, 2004), while Chu indicated the supplemental fermented agro by-products diet had no effect on concentration of leukocytes, erythrocytes, haemoglobin, total protein and triglycerides (Chu et al., 2011). Wang indicated XOS did not significantly improve swine serum antibody titres, IgA and IgM content, but can significantly increase the IgG (Wang, Pan, Shu-Yun, & Wang, 2006). In this study, there were no significant influences in the IgA, IgM, IgG and TP among groups.

Conclusions

In conclusion, the WSW could be well digested by Hu sheep with the amount of 2 kg/day/sheep. In the range of 2%, with the increase of the level, there was better F/G and CP digestibility. In the condition of this study, the optimum supplementation content of WSW for sheep is 2%. It provides a new strategy for recycling wheat starch processing water, avoiding environmental pollution.

Disclosure statement

No potential conflict of interest was reported by the authors.

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