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The effects of solid state fermented common vetch (*Vicia sativa*) with *Aspergillus niger* in broilers

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ABSTRACT: The effects of 20% raw vetch, solid state fermented vetch and enzyme added raw vetch addition in broiler diets on the performance, carcass characteristics, meat quality and digestibilty were investigated. Sixty-four one day old Ross 308 male broiler chicks were randomly divided into 4 groups each containing 16 animals. The diets of the groups were designed as control (C), 20% raw vetch added (RV), 20% solid state fermented vetch added (FV) and raw vetch added with enzyme (RVE). *Aspergillus niger* was used as a microorganism in the solid state fermentation of raw vetch seeds and fermentation continued for 72 hours. According to the findings obtained during the experiment that lasted for forty-two days; the fermentation process had no significant effect on the performance parameters for the entire production period compared to the use of raw vetch. When vetch was used as raw, fermented or enzyme addition, there was a tendency to increase the live weight of the groups at the 42.d (2607.46g, 2504.63g, 2645.23g). The use of vetch had a positive effect on breast meat weights (RV=757.00g, FV=747.27g and RVE=752.81g) and yields (RV=38.86g FV=40.42g and RVE=38.80g). Fermentation or enzyme addition to raw vetch did not affect the carcass parameters. The relative duedonum weight decrease with fermentation(65%) or using enzyme (67)%, also jejunum+ileum relative weights decrease in all vetch groups (RV=2.67, FV=2.46 and RVE=2.37). While the L* value of breast meat increased in the enzyme added raw vetch group addition (52.70), the a* value decreased in the fermented vetch (2.20). pH, nutrient values, breast meat thawing-cooking losses, water holding capacity and sensory analysis values in breast and tight meats were not affected by the treatments. Digestibilty of feeds did not affected in all treatments. According to these results, it can be recommended to use vetch at the level of 20% raw, fermented or enzyme addition in broiler diets without any negative effects.

Keyword: Feedstuffs, broilers, digestion, metabolism, meat.

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INTRODUCTION

Vetch (*Vicia sativa*) is a legume forage crop. It is in roughages class that can be evaluated as hay or straw, and when it comes to its grains, it is in the concentrates class. Vetch is cheap compared to other legume grain feeds and is a source of quality protein (Ergün et al. 2004). Huang et al. (2017) vetch contained an average of 90.6% dry matter, 28.3% crude protein, 1.5% ether extract and 5.0% crude fiber. However, its use in broiler diets is limited due to the anti-nutritional factors such as vicine, convicine, tannins and β-cyanoalanine it contains (Farran et al. 2001a). To eliminate the negative effects of these anti-nutritional factors on performance and health, it is necessary to apply some technological processes. These processes include soaking, boiling, steaming, treatment with organic acids etc. (Huang et al. 2017). Soaking vetch seeds for 24, 48 and 72 hours has been reported to significantly reduce these anti-nutritional factors (Farran et al. 2002). Farran et al. (2001b) reported that soaking the vetch seeds in water containing 1% $NaHCO_3$ or CH_3COOH reduced these substances. It has been determined that when cooked vetch seeds is used in broiler diets at 10% inclusion level, it does not make a significant difference in terms of live weight gain and feed conversion ratio (Darre et al. 1998). Sadeghi et al. (2011) in their study; reported that the addition of 10% of raw vetch seeds to diet did not make any significant difference, while the use of 20% had negative effects on the performance. However, they found that the use of 20% of soaked or cooked vetch seeds gave similar values as the control group; they stated that the use of 30% reduced the performance values. In another study, there was no significant effect on the live weight gain and carcass parameters diets contain 10% boiled or raw vetch seeds, but 30% had adverse effect on the performance of broilers (Saki et al. 2008). Sezmiş and Macit (2018) found that although 20% of soaked or roasted vetch decreased performance parameters and reduced the mortality rate compared to raw vetch.

Solid state fermentation is a fermentation process that occur at low humidity level different from deep state fermentation that occurs in liquid media. The substrate used in this process is a carbon/energy source that lacks free water or has low free water levels, and in some cases, additional hydration may be necessary (Pandey, et al. 2008). Therefore, fungi species are generally used as microorganisms. Solid state fermentation has been used since thousands of years ago to increase the nutrient content or taste, to facilitate its storage and to eliminate the effects of some harmful content of food (Krishna 2005; Singhania et al. 2009; Ahmed et al. 2014). Besides these, solid state fermentation has been used in the production of many bioactive substances such as enzymes, antibiotics, surfactants, biocides or biogas for a long time (Raghavarao et al. 2003). It is a method that can be used to improve the quality of animal feeds or to eliminate the effects of anti-nutritional factors (Lateef et al, 2008; Kaur et al, 2009). Fermentation has been shown to reduce anti-nutritional factors in animal feed. Solid state fermentation decreased glucosinolate content in canola meal by 38% (Ahmed et al. 2014). Fermentation or pre-digestion mitigated the negative immune effects of raw peas in broiler diets (Röhe et al. 2017) and improved turkey growth performance when combined with enzyme addition (Goodarzi-Boroojeni et al. 2018). Fermenting cottonseed meal reduced aflatoxin-related negative effects in duck diets (Liu et al. 2017). Additionally, fermented grains improved feed conversion ratio, final body weight, and carcass yield in broilers while reducing fecal viscosity (Yaşar, et al. 2016).

Aspergillus niger is a species of filamentous fungi that can produce various enzymes (such as hemicellulase, hydrolase, pectinase, lipase and tannase), which are widely used in solid state fermentation in the industry (fermented food, enzyme production, etc.), reduce the level of anti-nutritional factors, break down non-starch polysaccharides (NSP) and proteins (Honget al. 2004; Mathivananet al. 2006; Aderemi and Nworgu 2007; Dei et al, 2008b). The addition of 1% fermented (with *A. niger*) cherry inner seeds increased performance parameters like live weight and improved intestinal microflora in broilers (Güngör, 2018). The fermentation of the soybean meal with *A. niger* for 48 hours enhanced live weight, positively affected feed conversion ratio, and increased ileum villus length in broilers total period (Mathivanan et al, 2006). In a seperate study with *A. niger* fermented cassava roots improved energy metabolism in cockerels (Oso et al., 2015).

This study aimed to investigate the effects of the addition of 20% of raw vetch, solid state fermented vetch and raw vetch with enzyme to diets on the performance, carcass characteristics, meat quality and digestibility parameters of broilers chickens.

MATERIAL AND METHOD

Ethical statement

The experimental procedures for animal trials were approved by the Ege University Animal Ethics Committee (confirmation code 2020.074) and complied with the Turkish Code of Practice for the Care and Use of Animals for Scientific Purposes.

Experimental birds and housing, experimental diets and proximate analysis

Sixty-four one day old Ross 308 male broiler chicks were randomly divided into 4 groups each containing 16 birds. The birds housed as a group in the 1st week and were placed in individual metabolism cages at the beginning of the 2nd week. Feed and water were given *ad libitum* throughout the experiment; temperature and humidity values were adjusted according to the guide book; 23L:1D lighting program was applied. The study was conducted for 42 days. Chemical analysis of feedstuffs were made and diets were prepared for each group. Corn-soybean based diet was given to the control group (C). Raw vetch seeds (%20) were used in 2nd group (RV); 20% fermented vetch (FV) in 3rd group and multi-enzyme was used with 20% raw vetch (RVE) in group four. Starter diets were given 0 to 3 weeks and grower diets were given 3 to 6 weeks. Feedstuffs and experimental feeds were ground through a 1 mm screen in preparation for chemical analysis. Dry matter, crude protein, ether extract and crude fiber of the feedstuffs and diets were determined according to the Weende analysis methods (AOAC 1990). The starch and sugar content of the feeds was determined by Nauman and Bassler 1991. Estimates for metabolic energy (ME) were based on protein, ether extract, starch and sugar concentrations determined from the experimental diets (TS ISO 9610, 2020). Amino acid composition of the raw vetch and fermented vetch were determined at Ege University Central Research Test and Analysis Laboratory Application and Research Center. The nutrient makeup of raw vetch and fermented vetch are detailed in Table 1 and the experimental diets used are given in Table 2.

Microorganism and solid state fermentation

Aspergillus niger Van Tieghem, Anamorph (ATCC® 9142™) was used as inoculant in the study. *A. niger* spores were solid on potato dextrose agar (PDA). It was incubated at 24°C for 7 days. At the end of seven days, the petries were inverted and tapped for spore harvest; 1.8x10^7 CFU/ml solution was prepared with distilled water. Raw vetch seeds in 2 mm particle sized, mixed with the prepared solution (2:1). The mixture placed in nylon bags is closed so that air remains in it and left on a flat floor to be 4 cm thick. They are fermented for 72 hours at 30°C and they protected from the sunlight. At the end of this period, macroscopic mycelium was observed. The mixture was laid in metal containers with a thickness of 2 cm and dried at 50°C for 3 days. At the end of dry up processes, the nutrient composition of solid state fermented vetch seeds, which are turned into feed raw material, was determined.

Table 1. Proximate chemical composition of raw vetch and fermented vetch

Nutrients (%)	Raw Vetch	Fermented Vetch
Dry matter	90.20	91.00
Crude ash	1.2	1.4
Organic matter	89.00	89.60
Crude protein	29.54	31.14
Amino acids (%)		
Aspartic acid	0.264	0.229
Glutamic acid	0.609	0.635
Asparajine	0.024	0.029
Serine	0.000	0.000
Glutamine	0.015	0.010
Histine	0.000	0.000
Glisine	0.386	0.434
Threonine	0.018	0.029
Arginine	2.027	0.121
Alanine	0.208	0.368
Tyrosine	0.070	0.070
Cystine	0.044	0.032
Valine	0.164	0.178
Methionine	0.131	0.102
Norvaline	0.008	0.011
Tryptophan	0.225	0.124
Phenylalanine	0.094	0.113
Isoleucine	0.182	0.206
Leucine	0.361	0.434
Lisine	0.173	0.186
Hydroxsyprolin	0.230	0.271
Sarcozine	1.661	1.113
Prolyne	0.095	0.087
Ether extract	1.20	0.90
Crude fiber	4.88	4.41
Nitrogen free substances	53.38	53.11
Starch	34.1	29.3
Sugar	1.9	1.4
Metabolic energy (kcal/kg)	2612.77	2432.48

Table 2. The composition of starter diets at 0 to 3 weeks and grower diets at 3 to 6 weeks

Ingredients (%)	Starter diets				Grower diets			
	C	RV	FV	RVE	C	RV	FV	RVE
Maize	46.53	36.39	35.67	36.63	44.23	36.89	36.90	36.43
Wheat	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean meal	24.59	14.43	12.13	14.14	27.40	14.50	13.46	14.61
Full fat soya	12.00	12.00	15.00	12.00	14.00	14.70	15.00	14.70
Raw vetch	-	20.00	-	20.00	-	20.00	-	20.00
Fermented vetch	-	-	20.00	-	-	-	20.00	-
Fish meal	4.72	5.00	5.00	5.00	-	-	-	-
Sunflower seed oil	4.00	4.00	4.00	4.00	6.15	5.55	6.07	5.71
Lime stone	0.59	0.59	0.60	0.59	0.51	0.48	0.51	0.51
Dicalcium phosphate	1.58	1.58	1.59	1.59	1.95	1.88	1.96	1.99
Salt	0.17	0.19	0.22	0.19	0.22	0.24	0.22	0.25
Sodium bicarbonate	-	0.02	0.02	0.04	0.14	0.13	0.20	0.18
L-lisine	0.26	0.19	0.19	0.20	-	0.10	0.12	0.10
DL-metionine	0.21	0.20	0.20	0.20	0.16	0.21	0.21	0.21
L-treonine	0.10	0.16	0.13	0.16	-	0.04	0.04	0.04
Vitamin- mineral premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Multi-enzyme[2]	-	-	-	0.01	-	-	-	0.01
Nuritent Composition (%)								
Dry matter	89.67	89.92	89.87	89.72	89.67	89.67	89.90	89.71
Crude ash	5.34	5.40	5.21	5.15	5.01	4.80	4.97	4.83
Crude protein	23.05	23.31	23.59	23.64	21.90	21.75	21.72	21.75
Ether extract	8.53	8.47	9,03	8.47	10.61	10.14	10.66	10.28
Crude fiber	3.15	3,26	3.20	3.39	3.67	3.78	3.54	3.66
Starch	34.84	34.85	33.33	34.81	33.62	35.17	34.19	34.87
Sugar	3.34	2.84	2.77	2.81	3.67	3.04	2.88	3.04
Metabolic energy (kcal/kg)	3047	3037	3031	3046	3147	3135	3133	3135

C, control group; RV, raw vetch seeds (%20); FV, 20% fermented vetch and RVE, multi-enzyme was used with 20% raw vetch.[1] In 2.5 kg: 15000000 IU Vitamin A, 2000000 IU Vitamin D3, 300000 mg Vitamin E, 5000 mg Vitamin K3, 5000 mg Vitamin B1, 8000 mg Vitamin B2, 75000 mg Niacine, 18000 mg Calcium D Pantothenate, 5 200 mg Vitamin B6, 25 mg Vitamin B12, 250 mg D Biotine, 2000 mg Folic acid, 500000 mg Coline chloride, 80000 mg Manganese, 60 000 mg Iron, 60 000 mg Zinc, 5 000 mg Cooper, 200 mg Cobalt, 1000 mg Iodine, 235. [2] In 1.0 kg: Endo-1,3 (4)-beta glucanase 9000 U/g, Endo- 1,4- beta xylanase 43000, Pprotease 300000 U/g, Phytase 1000 FTU/g.

Performance parameters

In the experiment, live weights of birds were measured on days 0, 21 and 42. On the same days, the individual feed consumption of birds are determined (the group average for the first week is taken into calculations). From these values, live weight gain, feed intake and feed conversion ratio were calculated between 0-21, 22-42 and 0-42 days respectively.

Carcass and organs measurement

At the end of forty-two days, the birds were slaughtered to determine their carcass characteristics and samples were collected for meat quality analysis. Carcass, thigh, breast and abdominal fat weights of the slaughtered birds were weighed and carcass, thigh and breast yields were calculated by using slaughter weights. Proventriculus, gizzard, duodenum, jejunum+ileum, cecum, colon weights and heart, liver, pancreas, spleen, bursa fabricus weights of birds were measured and their relative weights were calculated.

Meat composition and quality

In all, 64 breast and thigh samples labeled were collected in plastic trays, weighed and stored in an air-tight plastic bags in freezer until the samples were required for analysis. Then they were homogenised using a blender and analysed for dry matter, crude ash, crude protein and ether extract according to the Weende analysis methods (AOAC, 1990). The pH values of breast meats were measured 15 minutes after slaughter with a pH meter with a meat probe attached (Hanna, USA). Meanwhile, L* (brightness), a* (redness) and b* (yellowness) values of breast meats were measured using color spectrophotometer (Konica-Minolta CR 410, Japan). Thaw and cook losses (TL and CL) were measured on the samples taken from the breast meat at slaughter (Honikel 1997). For these measurements, after freezing (-20^{o}C deep freezer), thawed and cooked (80^{o}C water bath/25 min.) weight measurements were used. The water holding capacity (WHC) of breast meat samples was measured (Chen et al. 2006). The meat weighing 0.25 mg was pressed under 140 kg/F pressure between 2 filter papers for 1 minute and re-weighed for this parameter. Right breast meat samples were minced through a 3 mm die, wrapped in film and stored at –25°C for up to one week for analyses of sensory properties. Panel members were trained to familiarise themselves with the properties to be evaluated. The following attributes were evaluated by the scoring test: aroma, softness, flavour, juiciness, appearance and overall acceptability. Samples were cooked in a grill at 250 °C for 3 min each side before serving to the panel. The sensory attributes namely aroma, softness, flavour, juiciness, appearance and overall acceptability were evaluated using an 5-point hedonic scale, where 1 = extremely undesirable and 5 = extremely desirable for the sensory attribute (TS ISO 3972, 2015). They were instructed to eat unsalted crackers and to drink water between each sample to clear their palate and pause for 20 s between samples.

Nutrient digestibility and chemical analysis of feces

Total feed intake was measured individually. Feed digestibility was measured from days 17-21 and 38-42 by total collection of excreta from each cage. Excreta were collected from individual birds, quantitatively daily. In all, 64 fresh well labeled excreta samples were weighed, and pooled within a cage. Pooled excreta were mixed well in a blender, and representative samples were obtained and dried. Dried excreta samples were ground to pass through a 1 mm sieve and stored in an air tight plastic bag in -20^{o}C until analysed for chemical composition. Dry matter, crude ash, crude protein and ether extract were determined according to the Weende analysis methods (AOAC 1990). In order to estimate protein digestibility, faecal and urinary N were chemically separated according to the method of Marquardt (1983). Digestibility was determined by accurately measuring feed intake and excreta output. From these measurements, together with chemical analysis for nutrients, the digestibility was calculated.

Statistical analysis

The study was conducted with 64 chicks. They were randomized to 4 groups (16 birds each group). SAS statistical package program was used to data evaluation (SAS Instute 1998). The evaluation of the data was made according to the one-way "ANOVA" procedure. The confidence interval is set at 95%. "Tukey Student T" test was applied to the differences between the means.

RESULTS AND DISCUSSION

Performance and carcass parameters

Performance parameters of the experimental groups have given at Table 3. Average livability value was % 100 for experiment and there were no treatment differences. The highest live weights values were observed in the FV (858.17g) and RV (858.08g) groups on day 21 ($P<0.05$). However, no differences were detected on live weight of the groups on day 42 ($P>0.05$). It is thought that these differences seen on day 21 are individual, these differences disappear when the animals grow up. Live weight gains were significantly higher in groups that consumed raw vetch and fermented vetch with enzyme during the finisher period ($P<0.05$). During this period, RVE (1897.70g) gave the highest value, followed by RV, C and FV ($P<0.05$). In whole production period, there were no statistically significant differences between the live weight gains of the groups ($P>0.05$). Goodarzi-Boroojeni et al. (2018), in a similar study using peas, concluded that live weight gains significantly increase in the control and multi-enzyme supplemented groups during the starter period, but that there was no difference between the groups during the whole production. Ashariyazadeh et al. (2018) reported that groups using fermented canola meal showed similar live weight gain as control, and nevertheless lower in raw canola meal. Wang et al. (2012) reported that the use of fermented soybean meal did not affect live weight gain over the entire production period which is similar to our study.

Feed intakes were higher in RV and PV (967.21g and 957.94g) than in other groups during the starter

Table 3. Performance parameters of the broilers

Group	C	RV	FV	RVE	SEM	P
Live weight (g)						
0 d	44.66	45.24	45.23	44.79	0.83	0.943
21 d	681.79[b]	858.08[a]	858.17[a]	747.52[ab]	40.34	0.005
42 d	2366.24	2607.46	2504.63	2645.23	94.44	0.164
Live weight gain (g)						
0-21 d	637.13[b]	812.83[a]	812.94[a]	702.72[ab]	40.32	0.005
22-42 d	1684.45[b]	1749.38[ab]	1646.45[b]	1897.70[a]	66.52	0.049
0-42 d	2321.58	2562.22	2456.26	2600.43	94.44	0.166
Feed intake (g)						
0-21 d	856.16[b]	967.21[a]	957.94[a]	845.74[b]	34.10	0.017
22-42 d	3312.56	3326.55	3232.95	3206.68	106.43	0.821
0-42 d	4166.72	4293.76	4190.89	4052.43	121.36	0.576
Feed conversion ratio (g/g)						
0-21 d	1.38	1.22	1.24	1.22	0.06	0.319
22-42 d	1.99[a]	1.93[a]	1.99[a]	1.68[b]	0.07	0.019
0-42 d	1.82[a]	1.69[ab]	1.73[a]	1.55[b]	0.05	0.020

C, control group; RV, raw vetch seeds (%20); FV, 20% fermented vetch and RVE, multi-enzyme was used with 20% raw vetch. [a,b] Within a column, values not sharing a common superscript letter are significantly different (P ≤ 0.05). SEM, Standard error of means (pooled).

period (P<0.05). In addition, no significant difference was detected between feed intake during the finisher period and the entire production (P>0.05). When the feed conversion ratios of the birds were examined, there was no statistically significant difference between all vetch groups and control group (P>0.05). In the finishing period, it is seen that RVE had better feed convertion ratio results (P<0.05). Aljubori et al. (2017) reported that the use of 30% fermented canola meal in days 1-28 adversely affected feed conversion, however this effect disappeared days 29-35. In 0-42 days period, fermentation of the vetch seed made no difference in feed conversion ratio compared to the raw vetch and control diet. Nonetheless the addition of enzymes improved the feed conversion ratio (P<0.05). Sezmiş and Macit (2018) applied processes such as soaking and roasting to vetch seed and they determined that these processes did not affect the feed conversion ratio in broilers. This result is similar to the groups other than the enzyme-added group in our study. In their study, Sadeghi et al. (2011) found that the use of raw vetch at the level of 20% did not make a difference in performance parameters compared to the control group. Nie et al. (2015) added raw and fermented cottonseed meal to slow-growing broilers diets and they found that the control group gave the best feed conversion ratio at finisher period. Also, Xiong et al. (2016) showed an increase in average daily live weight gain in groups using fermented cottonseed meal compared to control group in broilers. They also reported that the fermentation process increased this value compared to the raw cottonseed meal. While there was no difference in feed intake, the fermented cottonseed meal and control groups similarly determined a better feed conversion ratio than the raw cottonseed meal group. Ashayerizadeh et al. (2018); found that the use of fermented or raw canola meal in diets did not make an affect to live weight gain, feed intake and feed conversion ratio, but improved the feed conversion ratio compared to group that did not use canola meal. Dei et al. (2008a) found that the use of fermented sheanut meal in the diets gave better results in daily live weight gain and feed intake. However, they determined similarly higher results in the feed conversion ratio in the fermented group and control than in the raw group. Tang et al. (2012) reported that using fermented cottonseed meal cause the highest body weight gain in starter and finisher (control gave the lowest) periods. In the whole production period, only 8% substituted group gave the highest value in terms of live weight gain, but feed consumption and feed conversion ratio were not affected. Chukwukaelo et al. (2018) concluded that fermented cassava root and palm seed meal as replacement for maize affected the final body weight and feed conversion ratio was negatively. Goodarzi-Boroojeni et al. (2017) reported that the combined use of raw peas and enzyme in broiler chickens gave the best results in feed conversion ratio. When our study and the literature are compared, different or similar results are seen in the starter and finisher periods. These differences are thought to be due to different fermentation methods (microorganism, substrate, conditions, etc.) or usage rates.

The carcass parameters results have showed in Table 4. In 42nd day there are no statistically significant differences between carcass weights and yields obtained at slaughter (P>0.05). Breast weights and breast yields were significantly higher in all vetch-treated groups (the highest in RV) than in C (P<0.05). Although the highest leg weight (598.49 g) was detected in the RVE group (P<0.05), the differences between thigh yields were statistically insignificant (P>0.05). Abdominal fat weights and yields were similar to each groups (P>0.05). Nie et al. (2015) concluded that when they added raw or fermented cottonseed meal in diets; carcass, thigh and breast meat weights were not affected, but abdominal fat was the lowest in the control group. Mathivanan, Selvaraj and, Nanjappan (2006) reported that the addition of certain levels of fermented soybean meal and enzyme in diets did not affect carcass weights. According to Jazi et al. (2018), 10% raw and fermented cottonseed meal did not affect carcass weight, but reduced it by 20%, and determined that it did not make a difference in thigh and breast weights at both levels. Abdominal fat weight decreased in groups using fermented meal. Chukwukaelo et al. (2018), in their study where they used fermented cassava root and palm kernel meal instead of corn, found that carcass and abdominal fat

Table 4. Carcass parameters, relative weights of internal and gastrointestinal organs of the broilers

Group	C	RV	FV	RVE	SEM	P
Live weight(g)	**2366.24**	**2607.46**	**2504.63**	**2645.23**	**94.44**	**0.164**
Carcass parameters of the broilers						
Carcass weight (g)	1692.15	1922.69	1842.71	1944.68	70.99	0.067
Carcass yield (%)	66.71	67.55	69.16	68.51	0.70	0.091
Breast weight (g)	594.84^{b}	757.00^{a}	747.27^{a}	752.81^{a}	32.22	0.002
Breast yield (%)	34.94^{b}	38.86^{a}	40.42^{a}	38.80^{a}	0.97	0.002
Leg weight (g)	528.39^{b}	578.32^{ab}	526.21^{b}	598.49^{a}	20.90	0.042
Leg yield (%)	31.29	30.15	28.62	30.90	0.76	0.089
Abdominal fat weight (g)	26.94	27.32	20.94	28.56	3.06	0.312
Abdominal fat yield (%)	1.61	1.42	1.12	1.45	0.15	0.192
Relative weights of internal organs of the broilers						
Liver	2.52	2.77	2.59	2.76	0.08	0.078
Heart	0.82	0.76	0.74	0.79	0.03	0.433
Spleen	0.14	0.14	0.15	0.11	0.61	0.378
Pancreas	0.33	0.32	0.30	0.29	0.02	0.458
B. fabricus	0.14	0.21	0.17	0.16	0.023	0.212
Relative gastrointestinal organs weights of the broilers						
Proventriculus	0.63^{a}	0.54^{b}	0.51^{b}	0.51^{b}	0.026	0.004
Gizzard	2.49^{a}	1.92^{b}	1.97^{b}	1.92^{b}	0.08	<0.0001
Duedonum	0.79^{a}	0.79^{a}	0.65^{b}	0.67^{b}	0.03	0.008
Jejunum+Ileum	3.02^{a}	2.67^{b}	2.46^{b}	2.37^{b}	0.11	0.001
Ceacum	0.66	0.62	0.60	0.56	0.03	0.339
Colon	0.31^{a}	0.27^{ab}	0.24^{b}	0.23^{b}	0.021	0.04

C, control group; RV, raw vetch seeds (%20); FV, 20% fermented vetch and RVE, multi-enzyme was used with 20% raw vetch. ^{a,b} Within a column, values not sharing a common superscript letter are significantly different (P ≤ 0.05). SEM, Standard error of means (pooled).

weights decreased as the replacement rate increased, and breast meat weight decreased when 100% replacement was made.

Relative weights of internal organs and gastrointestinal organs

The relative weights of liver, heart, spleen, pancreas and bursa fabricus of the experimental groups (Table 4) were not affected by any treatment, and the differences between the averages were not statistically significant (P>0.05). Ashayerizadeh et al. (2018), reported that 50% and 100% soybean meal replaced with fermented or raw rapeseed meal in diets increased liver weights, the spleen was not affected and the weight of bursa fabricus decreased for the 10th and 22nd days. According to Xiong et al. (2016), fermented cottonseed meal and raw cottonseed meal increased the relative weights of the liver similarly but had no effect on the spleen and bursa fabricus relative weights. According to Tang et al. (2012) did not detect significant differences in spleen and bursa fabricus weights of broilers fed with diets include fermented cottonseed meal. According to Jazi et al. (2018) found that liver, heart, spleen and bursa fabricus weights were not affected when they added fermented and raw cottonseed meal to broiler diets. Since the relative weights of the internal organs did not change, it can be taken as an evidence that the addition of vetch did not put any pressure on them.

The relative gastrointestinal organs weights of the birds used in the experiment were found to be significantly lower in the all vetch groups compared to the control group, but only the relative duodenum weight of RV group was found to be similar to the control group (P<0.05), (Table 4). Mathivanan et al. (2006) concluded that the addition of fermented soybean meal or enzyme to the diet did not affect intestinal and pancreas weights. Gastrointestinal organ weights showed an overall reduction with vetch use. This suggests that the anti- nutrition factors in vetch (raw, fermented or enzyme) negatively affect the development of these organs. The weight of the duodenum was lower in the fermented and enzyme group, both of which are processes that would require this organ to less secretion.

Meat Quality

Our study results show that the addition of raw (with or without enzyme) and fermented vetch to the feed did not have a significant effect on the nutrients of the breast and thigh meat (Table 5) (P>0.05). Marcinčák et al. (2018) stated that the solid state fermented feeds did not make a difference in terms of dry matter and crude protein content in breast meat, but they reduced the ether extract content of the fermented feed. Nie et al. (2015) reported that breast meat ether extract levels decreased in the fermented cottonseed meal group and crude protein increased in the group using raw cottonseed meal. In the thigh meat, crude fat fell again in the fermented cottonseed meal group. In terms of crude protein, it was found to be lower than control in both groups. These differences between the study results may be related to the anti-nutritional factors in the raw material.

L* value of breast meat of birds fed with enzyme added diet (52.70) were found higher than other groups (P<0.05). The highest value in the a* value

Table 5. Nutrient composition of breast and thigh meats of broilers

Group	C	RV	FV	RVE	SEM	P
Breast Meat						
Dry matter	27.21	26.82	27.43	27.46	0.31	0.473
Crude ash	1.41	1.47	1.42	1.48	0.04	0.700
Crude protein	25.26	24.04	25.28	24.71	0.48	0.237
Ether extract	0.35	0.33	0.30	0.29	0.03	0.532
Thigh Meat						
Dry matter	25.45	25.22	24.67	25.47	0.39	0.452
Crude ash	1.35	1.32	1.29	1.41	0.05	0.379
Crude protein	19.89	19.52	19.36	19.57	0.28	0.588
Ether extract	3.06	3.48	3.20	3.32	0.021	0.549

C, control group; RV, raw vetch seeds (%20); FV, 20% fermented vetch and RVE, multi-enzyme was used with 20% raw vetch. SEM, Standard error of means (pooled).

was determined in the C (3.83) and the consumption of fermented vetch significantly reduced this value (2.20) (P<0.05). There was no difference between the groups in terms of the b* in breast meat (P>0.05). The thigh meat color values, they were not affected (P>0.05). There were no significantly differences for both of meat type pH values measured at 15 minutes after slaughter (P>0.05). Lee et al. (2010), in their studies with germinated and fermented soybean meal, could not detect any differences in color values differently from our study, but they found similar results for pH values (Table 6).

The sensory analysis (Table 7) and thawing loss, cooking loss and water holding capacity (Table 8) of the breast meats we could not determine a statistically significant difference between the parameters (P<0.05). Marcinčák et al. (2018), found the similar results for these parameters with our results. Thus, it can be considered that the content of vetch does not

Table 6. Meat color values (L*, a* and b*) and pH (15 minute after slaughter).

Group	C	RV	FV	RVE	SEM	P
Breast meat						
L*	49.03^{b}	50.31^{b}	50.26^{b}	52.70^{a}	0.82	0.025
a*	3.83^{a}	3.67^{ab}	2.20^{c}	2.91^{bc}	0.30	0.002
b*	7.46	7.18	7.84	7.27	0.48	0.778
pH15	6.56	6.43	6.52	6.49	0.05	0.298
Thigh meat						
L*	49.60	51.88	49.20	52.04	0.92	0.067
a*	4.13	3.10	3.54	3.07	0.29	0.056
b*	7.70	6.90	7.52	7.04	0.55	0.709
pH15	6.55	6.51	6.36	6.55	0.07	0.200

C, control group; RV, raw vetch seeds (%20); FV, 20% fermented vetch and RVE, multi-enzyme was used with 20% raw vetch. L*measures relative lightness, a*relative redness and b*relative yellowness. [a,b] Within a column, values not sharing a common superscript letter are significantly different (P ≤ 0.05). SEM, Standard error of means (pooled).

Table 7. Sensory analysis of the breast meats

Group	C	RV	FV	RVE	SEM	P
Aroma	3.14	3.85	3.57	3.85	0.36	0.475
Softness	4.14	4.00	4.28	4.00	0.34	0.475
Flavour	3.71	4.14	3.85	4.00	0.33	0.464
Juiciness	3.71	3.42	3.14	3.42	0.35	0.464
Appearance	3.57	3.85	4.00	4.00	0.38	0.839
Overall acceptability	3.57	4.14	4.00	4.00	0.29	0.557

C, control group; RV, raw vetch seeds (%20); FV, 20% fermented vetch and RVE, multi-enzyme was used with 20% raw vetch. SEM, Standard error of means (pooled).

Table 8. Thaw loss, cook loss and water hold capacity of breast meats

Group	C	RV	FV	RVE	SEM	P
Thaw loss	5.73	5.87	5.69	5.83	0.72	0.997
Cook loss	15.70	15.92	16.95	16.90	0.85	0.637
Water hold capacity	22.79	23.43	23.11	22.67	0.46	0.665

C, control group; RV, raw vetch seeds (%20); FV, 20% fermented vetch and RVE, multi-enzyme was used with 20% raw vetch. SEM, Standard error of means (pooled).

have any effect on meat quality. This is a positive result in that the anti-nutritional factors in vetch do not negatively affect the market value of the meat.

Nutrient digestibility of the experimental diets

The results on nutrient digestibility Table 9 showed that organic matter (OM), crude protein (CP) and ether extract (EE) digestibility of the starter and finisher diets were evaluated, no statistically significant differences were found between the groups (P>0.05). Despite the decrease in small intestine weight, nutrient digestibility did not change with the use of vetch. This can be explained by morphological (increased villus absorption capacity) and enzymatic (increased secretion) adaptation in the small intestine (Tappenden, 2014). Dei et al. (2008b) in their studies using fermented shea nut found no difference in nutrient digestibility. Bartkiene et al. (2015) found that in vitro studies solid state fermentation increased crude protein digestibility in lupine and soybean. Goodarzi-Borojeni et al. (2018), in their study in which they added enzyme to raw and fermented peas and raw peas, revealed that the addition of enzyme gave better crude protein digestibility than other groups. It is a positive result that anti-nutritional factors did not have a negative effect on digestibility with the addition of vetch to the feeds.

CONCLUSIONS

The result of the study showed that raw or fermented vetch could be used up to 20% inclusion levels for improved performance, The fermentation process, when considered together with the use of raw vetch, does not have positive or negative effects on the performance parameters for the production period. However, the use of raw vetch with enzyme was positively affected the feed conversion ratio. The use of all vetch forms had a positive effect on breast meat weight and yield. The relative weight of the duodenum, which is also an enzyme-secreting organ, decreased by the use of enzymes or the effect of the fermentation process. Small intestine weight decreased with vetch use. Except for the meat color values, any meat quality parameters did not affected in all treatments. The nutrient digestibility similar in all groups.

The results obtained from our study are evaluated, based on the fact that no statistically significant difference is detected in the performance parameters except feed conversion ratio. It may be recommended to use raw vetch broiler diets at the level of 20% without any decrease in performance. Although no statistically significant differences have been identified, it is evaluated that the addition of enzymes to the raw vetch may increase the performance somewhat when evaluated numerically.

Future research requires the investigation of different enzyme combinations and levels. In addition, considering that solid state fermentation can be applied in many different ways, it is recommended to evaluate different solid state fermentation methods and different microorganisms.

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Table 9. Nutrient digestibility of the experimental starter and finisher diets.

Group	C	RV	FV	RVE	SEM	P
Starter Diets						
Organic matter	83.78	83.79	84.40	84.44	0.69	0.841
Crude protein	83.61	83.30	84.94	84.44	0.82	0.487
Ether extract	87.41	88.41	87.42	88.94	0.70	0.333
Finisher Diets						
Organic matter	81.11	82.58	84.92	85.07	2.34	0.577
Crude protein	74.97	75.45	79.01	78.51	3.25	0.749
Ether extract	87.38	89.52	89.12	91.30	1.54	0.366

C, control group; RV, raw vetch seeds (%20); FV, 20% fermented vetch and RVE, multi-enzyme was used with 20% raw vetch. SEM, Standard error of means (pooled).

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