Europ.Poult.Sci., **82.** 2018, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2018.222

Sourdough works as growth enhancer in quail (*Coturnix coturnix Japonica*)

Verwendung von Sauerteig als Wachstumsförderer bei Wachteln (*Coturnix coturnix japonica*)

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Manuscript received 12 December 2017, accepted 29 December 2017

Introduction

Bacteria population in the gut mucosal area affects gut health and consequently growth performance. Probiotics have been used to support gut health by increasing beneficial bacteria populations against pathogenic bacteria in gut. There have been a large number of studies on investigation the effects of probiotics in poultry. Successful probiotic effects in poultry has been found using *Bacillus spp* (Abdel-Hafeez et al., 2017; Gadde et al., 2017; Guo et al., 2017; Midilli et al., 2008), probiotic mix (Bozkurt et al., 2009; Khatun et al., 2017; Ayaşan, 2013), *E. faecium* (Samli et al., 2007) and *Lactobacillus spp* (Wang et al., 2017). Although there are a lot of studies including probiotics in literature, probiotic effects of different food additives including lactic acid bacteria and *Saccharomyces cerevisiae* need further investigation. Sourdough is one of those food additives. It is a starter bacteria culture for homemade bread and dates back in ancient times (Yagmur et al., 2016). Basically, full wheat flour and water mixture are used for sourdough making and fermentation begins after one day. Lactic acid bacteria (LAB), yeasts and *enterobactericaea* develop in sourdough at the beginning of the fermentation. Lactic acid bacteria and yeasts dominate the sourdough bacterial microflora with day by day refreshment (Minervini et al., 2014). Şimşek et al. (2006) reported that there are different forms of sourdough and the number of bacteria in the sourdough varies by region and form. Yagmur et al. (2016) determined 16 different lactic acid bacteria species and 5 yeast species in sourdough provided from 5 different bakeries having a pH of 4. They contained on average 8–9 log cfu/g lactic acid bacteria and 6–7 log cfu/g yeast. It is easy to produce sourdough, because only flour and water are sufficient for making sourdough and bacterial development (Yagmur et al., 2016). It can be produced by three different protocols, (a) traditional production with daily refreshment, (b) liquid production, and (c) dried production (Gobbetti, 1998; Corsetti and Settanni, 2007; Minervini et al., 2014). All of them are a good lactic acid bacteria and yeast sources. Although the sourdough is a good source of LAB and yeast, there has been no study on probiotic efficacy of liquid or dried sourdough for animal nutrition. It has been questioned whether sourdough can be used as a feed additive. Therefore, the aim of this study was to determine the effects of liquid and dried sourdough supplementation into drinking water and diet respectively on quail growth performance, ileum histomorphology and caecum microbiota.

Material and Methods

Animals and Housing

One hundred and twenty mixed-sex quail aged 14 d were used in this study and the trial lasted for 21 days. The quail were divided into three experimental groups and four replicates (10 chicks, 5 males and 5 females) at equal body weights. Experimental groups were as follows: control (C); dried sourdough (5 g/kg) for dietary supplementation (D); liquid sourdough (4 ml/l daily) for water supplementation (L).

All chicks in this study were housed in floor pens with wood shavings. They were fed *ad libitum* and offered fresh water daily with plastic waterer seized 1 l. The experimental diet was prepared according to recommendations of the National Research Council (NRC, 1994) (Table 1). Illumination was provided during the experiment. Room temperature was 33°C at the beginning of the study and then gradually decreased to 26°C on d 21 of the trial. Body weight and feed intake were measured at 0, 7, 14, and 21 d of study. At 21 d of study, two birds were slaughtered (one female and one male per replicate), to determine the weights of internal organs (heart, liver and gizzard) and proventriculus, gastrointestinal tract length (GITL), and ileum histomorphology. Mortality was not calculated since there was no death in any treatments.

Table 1. Composition of the experimental diet (as-fed basis)

Zusammensetzung der Versuchsration (Angaben in Frischsubstanz)

Feed Ingredients	g/kg
Maize	440
Soybean meal (44%)	412
Meat and bone meal (45%)	40.0
Soybean oil	65.0
Dicalciumphosphate	25.0
L-lysine HCl	7.0
DL-methionine	3.5
NaCL	3.0
Vitamin Premix*	2.5
Mineral Premix#	2.5
Analyzed nutrient composition	
ME [MJ/kg feed]	12.9
Crude protein	224
Crude fibre	28.0
Crude fat	85.0
Calcium	76.0
Available phosphorus	38.0

* Premix provided per kg of diet: * Vitamin A, 12000 IU; Vitamin D3, 2400 IU; Vitamin E, 30 mg; Vitamin K_3, 4 mg; Vitamin B1, 3 mg; Vitamin B_2, 7 mg; Vitamin B_6, 5 mg; Vitamin B_{12}, 15 µg; niacin, 25 mg; # Fe, 80 mg; folic acid, 1 mg; pantothenic acid, 10 mg; biotin, 45 mg; Choline, 125000 mg; Cu, 5 mg; Mn, 80 mg; Zn, 60 mg; Se, 150 µg.

Preparation of dried and liquid sourdough

A total of 500 g full wheat flour and 500 ml naturel water were mixed for stock material and incubated in open glass bottles at 30°C for two days in an incubator. After two days, half of the stock material was mixed with 500 g dough (50% wheat flour and 50% water) every day for 8 days. At the end of the 10 days, pH: 3.8, LAB: $8.3 * 10^8$ and Yeast $6.4 * 10^6$ were determined in the stock material.

Liquid sourdough was prepared with 10% sourdough solution for daily water supplementation. Liquid sourdough (4 ml) was injected into the drinking water via a 10 ml injector to each L group for every day.

Dried sourdough was prepared with 500 g stock material mixed with 500 g tiny granulated wheat for fast dry at room temperature for 2 days. Dried sourdough was supplemented with 5 g/kg to the experimental diet.

Caecum microbiota

Samples of the caecal contents were collected into sterile glass tubes in which they were kept on ice until subsequent inoculation into agars. MRS agar (MERCK, Darmstadt, Germany, 1.10660) was used for enumeration of lactic acid bacteria (LAB) at 37°C for a 3-d incubation period and malt extract agar (MERCK, Darmstadt, Germany, 1.05398) was used for enumeration of yeast at 30°C for a 3-d incubation period. VRBD (Violet Red Bile Dextrose) (MERCK, Darmstadt, Germany, 1.01406) agar was used for enumeration of *Enterobacteriaceae* at 37°C for an 18 – 20 h incubation period. 3 M Petrifilm TM (3 M Microbiology Products St. Paul MN 55114 USA) was used to determine *Escherichia coli* and *Coliformes* count in caecal samples. The following manufacturer's instructions for incubation conditions were used to determine the microbial counts of samples: *Escherichia coli*: at 32°C for 24 h; *Coliformes*: at 35°C for 24 h.

Bacterial colonies were counted by determining the average number of live bacteria per g caecal content. LAB, *Yeast*, *Enterobacteriaceae*, *E. coli* and Coliforme bacteria counts of the samples were converted into logarithmic colony forming units (cfu g^{-1}).

Ileum histomorphology

Ileum samples were cut into 1.0 cm pieces and placed into 10% formalin for histomorphological processing. Tissues sections were inserted into tissue cassettes. After the dehydration process, tissue sections were embedded in paraffin blocks, cut to 10-µ thickness, and placed on a slide. Each sample of ileum histomorphological tissue was prepared and stained with PAS staining procedure by using standard paraffin-embedding methods. After the embedding process, villi length and width were evaluated by using an image processing and analysis system (ZEN 2012 SP2) for Zeiss Primo Star HD Light Microscope.

Statistical analyses

The data were analysed using the ANOVA procedure of SPSS software (SPSS 15). Differences between group means were separated by Duncan's multiple range tests.

Results

Dietary sourdough supplementation increased live weight gain (LWG) in Japanese quail compared to control group (P>0.05), but liquid sourdough did not affect live weight gain as seen in Table 2. Similarly to live weight gain, feed conversion ratio (FCR) was better in the dietary sourdough supplemented group than in the control group (P>0.05). Feed intake was not affected by any treatments. The effects of dried and liquid sourdough on body parts are given in Table 3. Dietary or liquid sourdough supplementation did not affect relative weights of heart, gizzard, edible inner organs (heart+ gizzard+ liver), proventriculus and gastro intestinal tract length. Liver weight was decreased with dietary sourdough supplementation. The effects of dried and liquid sourdough on caecum microflora are given in Table 4. Dietary or liquid sourdough supplementation did not affect lactic acid bacteria (LAB), pathogenic *Enterobactericaea*, *E. coli* and *Coliformes* in caecum. *Saccharomyces cerevisiae* (SC) colonisation in caecum increased by dietary and liquid sourdough supplementation. While LAB colonisation in caecum tended to increase with dietary sourdough supplementation, pathogenic *Coliformes* count in caecum tended to decrease. Ileum histomorphological parameters are given in Table 5. Villi length increased in dietary and liquid supplemented groups compared to that of the control group. Dietary sourdough increased villi length much more than liquid supplementation. Villi width increased only by dietary supplemented group compared to control and liquid supplemented groups.

Table 2. Effects of dried and liquid sourdough on quail performance parameters

Einfluss von ‚trockenem‘ und ‚flüssigem‘ Sauerteig auf die Leistung der Wachteln

	C	D	L	SEM	*P*-values
LWG (g)	65.4b	69.2a	66.7ab	1.21	0.05
FI (g)	214	201	215	5.61	0.185
FCR	3.27b	2.90a	3.24ab	0.09	0.001

a-b – Means in a row with no common superscript letters differ significantly ($P<0.05$). LWG = Live weight gain, FI = Feed intake, FCR = Feed conversion ratio, C = Control, D = Dried sourdough, L = Liquid sourdough, SEM = Standard error of means.

Table 3. Effects of dried and liquid sourdough on relative weights of inner organs

Einfluss von ‚trockenem' und ‚flüssigem' Sauerteig auf die relativen Gewichte der inneren Organe

	C	D	L	SEM	*P*-values
Heart[1](g)	0.90	0.81	0.95	0.05	0.51
Liver[1](g)	3.56ab	3.00b	3.95a	0.15	0.03
Gizzard[1](g)	4.48	4.54	4.42	0.27	0.98
EIO[1](g)	8.94	8.36	9.32	0.31	0.46
GITL[2](cm)	50.3	57.2	53.2	2.36	0.52
Proventriculus[1](g)	0.60	0.58	0.57	0.04	0.97

[a-b] – Means in a row with no common superscript letters differ significantly (P<0.05). EIO = Edible inner organs (Heart + Liver + Gizzard). GITL = Gastro intestinal tract length, C = Control, D = Dried sourdough, L = Liquid sourdough, SEM = Standard error of means. 1) g/100 g live weight, 2) cm/100 g live weight.

Table 4. Effects of dried and liquid sourdough on caecum microflora

Einfluss von ‚trockenem' und ‚flüssigem' Sauerteig auf die Zusammensetzung der Mikroflora in den Blinddärmen

	C	D	L	SEM	*P*-values
LAB	6.38	6.80	6.11	0.24	0.57
Yeast	5.74b	7.06a	6.90a	0.23	0.001
Enterobacter	6.84	6.99	6.65	0.26	0.89
E. coli	7.02	6.94	7.01	0.20	0.97
Coliformes	7.74	6.80	6.77	0.35	0.49

[a-b] – Means in a row with no common superscript letters differ significantly (P<0.05). C = Control, D = Dried sourdough, L = Liquid sourdough, SEM = Standard error of means.

Table 5. Effects of dried and liquid sourdough on ileum histomorphology

Einfluss von ‚trockenem' und ‚flüssigem' Sauerteig auf die Dünndarm-Morphologie

	C	D	L	SEM	*P*-values
VL(µ)	195c	273a	231b	9.87	0.001
VW (µ)	36.1b	54.2a	40.2b	2.64	0.001

[a-b] – Means in a row with no common superscript letters differ significantly (P<0.05). C = Control, D = Dried sourdough, L = Liquid sourdough, SEM = Standard error of means, VL = Villi length, VW = Villi width.

Discussion

The results showed that dietary sourdough supplementation worked as growth promoter in Japanese quail due to increased villi length and width and increased counts of beneficial bacteria such as LAB and Yeast. Dietary sourdough supplementation increased live weight gain compared to the control group (P>0.05). The current study is the first record of dietary sourdough supplementation on livestock performance. In this study, 7.8 log cfu LAB and 6.7 log cfu yeast provided via 4 cm^3/l drinking water to W group and 8.9 log cfu LAB and 6.81 log cfu yeast provided via 0.5% supplemented diet (D) for each chick during the trial. It was determined that LAB and yeast in sourdough worked like probiotic mixture in Japanese quail. Also YASAR and YEGEN (2017) reported that a *Saccharomyces cerevisiae* fermented food additive enhanced broiler growth and increased feed conversion ratio. The results of this study corroborates similar earlier studies supplementing probiotics to livestock diets. Namely it was reported that dietary probiotics promoted live weight gain and developed FCR (ABDEL-HAFEEZ et al., 2017; BOZKURT et al., 2009; GADDE et al., 2017; GUO et al., 2017; KHATUN et al., 2017; MIDILLI et al., 2008; SAMLI et al., 2007; WANG et al., 2017).

It was determined that dietary sourdough supplementation decreased liver weight. BOZKURT et al. (2009) and ANJUM et al. (2005) reported that liver weight decreased with dietary probiotic containing LAB supplementation. The lower liver weight in dietary sourdough supplemented quail may be a result of more locomotor activity and a better health situation. Evidently, KALAVATHY et al. (2003) reported that dietary symbiotic (lactobacillus) supplementation decreased liver cholesterol level. SAHIN (1998) reported that a heavy liver occupies more space in the chest cavity and limits the lungs and reduces oxygen for metabolism of ingested nutrients. TEETER and BELAY (1996) reported that the amount of oxygen needed for protein synthesis is 4 times bigger than the amount for fat synthesis. Therefore, the lower liver weight can be indicator of more active and healthy life.

The present findings are the first published record in animal studies with respect to the efficacy of dried or liquid sourdough on caecum microbiota. The studies on supplemented dietary probiotics (LAB and SC) on broiler caecum microbiota are contradictory. BLAJMAN et al. (2017) reported that dietary *lactobacillus* as probiotic supplementation increased broiler performance without affecting LAB, *E. coli* and *Enterobactericaea* count in caecum. On the contrary, WANG et al. (2017) found that dietary probiotic (*Lactobacillus johnsonii*) supplementation increased both LAB count in ileum and increased broiler performance. Further, CHEN et al. (2017) reported that dietary probiotic (LAB) supplementation did not affect broiler performance but increased caecal LAB colonisation. These contradictory results may be explained by different factors like sanitation of experimental field, stress factors in experiments or litter material quality. OZTURK and YILDIRIM (2004) reported that broiler performance depends on multiple factors such as genetic, environmental or stress factors.

Like the microbiota results, dietary sourdough improved villi morphology better than a liquid solution. In the current study, increasing villi length and villi width depend upon dietary sourdough supplementation has been proposed to be an indicator of widened transport area and enhanced nutrient absorption (WANG et al., 2017; Samlı et al., 2007; YASON et al., 1987).

Conclusions

1) Dietary sourdough showed a probiotic effect by increasing quail growth performance, affecting caecum microflora and increasing ileum villi length.
2) Since sourdough production is easy, small-scale farms can easily produce it themselves when they cannot provide probiotics or other probiotic feed additives.
3) This is the first study on a homemade probiotic feed additive, suggesting that further studies should be carried out by using different animal species or different ingredients.

Acknowledgements

This study was conducted by using the Facilities of Agriculture Faculty of Ahi Evran University, Kırşehir, Turkey. The practices and procedures for this experiment were reviewed and approved by the local Animal Ethics Committee (02/20/2017–03). Author thanks to MsC students Figen Saydut, Kubra Sacihan and Abdurrahman Korkmaz due to their technical help during experiment.

Summary

This study was conducted to determine the effects of liquid or dietary supplementation of sourdough on quail performance, composition of caecal microbiota and ileum histomorphology. A total of 120 healthy 14 d old quail (*Coturnix cot. Japonica*) were divided into three dietary treatments with 4 replicates each including 10 mixed sex quail (5 males and 5 females). Treatments were 1) Control, 2) liquid sourdough (4 ml/l) in drinking water, 3) dried sourdough (5 g/kg) in experimental diet. The experiment lasted 21 days. Live weight gain (LWG), feed intake (FI), and feed conversion ratio (FCR) recorded weekly. Caecum microbiota and ileum histomorphology were determined at the 21st day of the experiment.

Results showed that the dietary sourdough supplementation increased LWG and FCR ($P<0.05$). FI was not affected by any treatments. Dietary sourdough supplementation decreased liver weight ($P<0.05$). Both dietary and liquid sourdough supplementation increased the colonisation from 5.74 to 7.60 and 6.90 cfu g^{-1} of yeast in the caecum respectively ($P<0.05$). Lactic acid bacteria (LAB) count tended to increase without any statistical difference. The colonisation of Enterobactericaea, *E. coli* and Coliforme bacteria were not affected by any treatments, but Coliformes counts tended to decrease. Dietary sourdough supplementation increased villi length and villi width compared to control and liquid sourdough supplemented group ($P<0.05$). To conclude, sourdough showed a probiotic effect and enhanced growth performance by affecting caecum microflora and increasing ileum villi length when given with the diet. Further studies with different animal species and different methods of application are needed.

Key words

Quail, nutrition, sourdough, feed additive, performance

Zusammenfassung

Verwendung von Sauerteig als Wachstumsförderer bei Wachteln (*Coturnix coturnix japonica*)

Im Rahmen der Studie sollte der Einfluss der Zulage von Sauerteig zum Trinkwasser oder zum Futter auf die Leistung, die Zusammensetzung der Blinddarmflora und der Morphologie des Dünndarms bei Wachteln untersucht werden. Hierzu wurden 140 gesunde, 14 Tage alte Japanische Wachteln (*Coturnix cot. japonica*) auf drei Behandlungsgruppen mit je 10 Wiederholungen verteilt. Eine Wiederholung umfasste 5 Hähne und 5 Hennen. Folgende Behandlungsgruppen lagen vor: 1) Kontrolle, 2) Zusatz von 4 ml/l flüssigem Sauerteig zum Trinkwasser, 3) Zusatz von 5 g/kg trockenem Sauerteig zum Futter. Die Versuchsperiode umfasste 21 Tage, die Lebendgewichtszunahmen (LWG), die Futteraufnahme (FI) und die Futterverwertung (FCR) wurden wöchentlich ermittelt. Die Zusammensetzung der Blinddarm-Mikroflora und der Darm-Morphologie wurden am 21. Versuchstag bestimmt.

Die Zulage von Sauerteig führte zu höheren Lebendgewichtszunahmen und einer günstigeren Futterverwertung ($P<0,05$). Die Futteraufnahme wurde dagegen durch die Behandlungen nicht beeinflusst. Die Zulage von Sauerteig verminderte das Lebergewicht ($P<0,05$). Der Einsatz von Sauerteig im Futter und im Trinkwasser erhöhte die Anzahl an Hefekolonien in den Blinddärmen gegenüber der Kontrolle von 5,74 auf 7,60 bzw. 6,90 ($P<0,05$). Die Anzahl an Milchsäure-produzierenden Bakterien (LAB) nahm nur tendenziell zu. Der Zusatz von Sauerteig wirkte sich nicht auf die Besiedelung der Blinddärme mit Enterobakterien, *E. coli* und koliformen Bakterien aus, allerdings wurden tendenziell höheren Anzahlen an koliformen Keimen beobachtet. Die Zulage von Sauerteig zum Futter führte im Vergleich zur Kontrolle und zur Zulage von Sauerteig zum Trinkwasser zu längeren und breiteren Villi im Darm ($P<0,05$). Es wurde der Schluss gezogen, dass Sauerteig generell ein probiotisches Potenzial besitzt und bei Zulage zum Futter die Wachstumsleistung von Wachteln, über die Veränderung der Mikroflora in den Blinddärmen sowie der Darmmorphologie, verbessern kann. Es sollten daher weitere Untersuchungen mit verschiedenen Zulagenverfahren und Geflügelspezies durchgeführt werden.

Stichworte

Wachtel, Fütterung, Sauerteig, Zusatzstoff, Leistung

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