PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 11 (3): 203-207, 2012
ISSN 1680-5194

Improving the Nutrient Quality of Juice Wastes Mixture Through Fermentation by Using *Trichodherma viride* for Poultry Diet

Yose Rizal[1], Maria Endo Mahata[1], Indra Joli[1] and Guoyao Wu[2]
[1]Faculty of Animal Science, University of Andalas, Padang, West Sumatra, Indonesia
[2]Department of Animal Science, Texas A&M University,
2471 TAMU College Station, Texas, 77843-2471, USA

Abstract: Two experiments were conducted to improve Juice Wastes Mixture's (JWM's) nutrient quality by using *Trichoderma viride* and to compare between treated vs. untreated one. This JWM consists of carrot, apple, mango, avocado, orange, melon and tree-tomato in the same proportion. Experiment 1 was performed in a 3 x 5 factorial experiment of completely randomized design with 3 replicates. First factor was *Trichoderma viride* concentration (5, 7 and 9%) and second factor was incubation period (0, 5, 7, 9 and 11 days). Measured variables were Crude Fiber (CF), Crude Protein (CP) and Ether Extract (EE). Experiment 2 was to compare nutrient content between treated vs. untreated one. Measured variables were JWM's CF, CP, EE, NDF, ADF, cellulose, hemi-cellulose, lignin, nitrogen retention, amino acid profile and Metabolizable Energy (ME). There was a very significant ($p<0.01$) interaction between *Trichoderma viride* concentration and incubation period in which 7% *Trichoderma viride* and 5 day incubation period reduced CF and EE and increased CP content. CF, EE, NDF, ADF, cellulose, hemicelluloses and lignin contents between untreated vs. treated one declined from 17.10, 6.24, 34.30, 24.40, 12.20, 9.90 and 11.80% to 12.23, 3.72, 31.55, 22.43, 11.15, 9.12 and 11.28%, respectively. Meanwhile, nitrogen retention and CP increased from 58.40 and 8.40% to 63.64 and 11.29%, respectively. ME content of treated was better than untreated JWM (1774 vs. 2599 kkal/kg). *Trichoderma viride* treatment improved JWM's amino acids profile. In conclusion, fermentation by 7% *Trichoderma viride* at 5 day incubation period was the best treatment for improving JWM's nutrient quality.

Key words: Juice wastes mixture, crude fiber, fermentation, *Trichoderma viride*, nutrient quality, poultry

INTRODUCTION

Diversification of feeds is one of the alternative solutions to overcome the shortage of feed, especially corn in poultry diets. This diversification can be obtained from different kinds of agricultural by-products or wastes, for example, the mixture of juice wastes of variety fruits and other food-stuffs. Some experiments about the utilization of wastes from fruits and others have been reported. Ghazi and Drakhshan (2006) reported that up to 15% inclusion of untreated tomato pomace could be recommended for practical poultry diet formulation. While, Al-Betawi (2005) reported tomato pomace can be used in broiler rations at a level of 10% from the total diet and more investigations are still needed on this subject. Olive pulp can also be included up to 7.5% in the ration of broilers (Rabayaa *et al.*, 2001). Zafar *et al.* (2005) found that the apple by-product could be used in broiler diets. Oluremi *et al.* (2006) reported that the dried sweet orange rind could also be used to replace dietary maize in broiler diets at the 15% level. Diarra *et al.* (2010) recommended that the boiled manggo kernel meal could replace up to 60% of the maize in the diet of broilers without adverse effects on growth, carcass measurements and blood parameters. The experiment on the utilization of carrot (*Daucus carotta*) juice waste and fruits [apple (*Mallus sylvestris*), manggo (*Mangifera indica*), avocado (*Persea americana*), orange (*Citrus* sp.), melon (*Cucumis melo L)* and tree potato (*Cyphomandra betacea* Sendtn.)] juice wastes mixture in the same proportion indicated that this juice wastes mixture could be included up to only 20% for the broiler diet to effectively replace 40% corn (Rizal *et al.*, 2010). The problem in the utilization of this juice wastes mixture is the high in its crude fiber content (17.1%), meanwhile, the concentration of crude protein (8.4%) of this juice wastes mixture (Rizal and Mahata, 2009) was similar to crude protein (8.5%) of corn (NRC, 1994). Further analyses for the amino acids contents of this juice wastes mixture encounted that triptopan was 4 times higher than of corn, lisin 1.6 times, glycine 1.6 times and threonine 1.3 times (Rizal and Wu, 2009). The comparison of amino acids contents of the carrot and fruits juice wastes mixture to corn is depicted in Table 1. Fermentation is one of several solutions to decrease the fiber content of the feed. Fungi of the species of *Trichoderma viride* is often used for fermenting feeds. This fungi produces several enzymes such as protease, lipase, pectinase and cellulase (Pelczar and Reid, 1974; Wiseman, 1981; Rogers, 2002). Utilization of *Trichoderma viride* decreased the crude fiber, NDF, ADF,

Corresponding Author: Yose Rizal, Faculty of Animal Science, University of Andalas, Padang, West Sumatra, Indonesia

Table 1: The comparison of amino acid content in carrot and fruit juice wastes mixture to corn

Amino acids	Amino acids concentration (%)	
	Corn[1]	Juice wastes mixture[2]
Aspartate	-	0.71
Glutamate	-	0.98
Serine	0.37	0.46
Histidine	0.23	0.14
Glycine	0.33	0.54
Threonine	0.29	0.39
Arginine	0.38	0.37
Alanine	-	0.54
Tyrosine	0.30	0.43
Methionine	0.18	0.13
Valine	0.40	0.44
Phenylalanine	0.38	0.37
Iso Leucine	0.29	0.34
Leucine	1.00	0.54
Lysine	0.26	0.42
Proline	-	0.68
Cysteine	0.18	0.05
Tryptophan	0.06	0.23

[1]NRC (1994). Nutrient Requirements of Poultry.
[2]Rizal and Wu (2009)

cellulose and hemicellulose content of cassava leaves (Rizal *et al.*, 2006b). There is no information available on the utilization of *Trichoderma viride* for reducing the fiber content of the juice waste mixture.

MATERIALS AND METHODS

Experiment 1: The main objective of this experiment 1 was to study the improvement of the nutrient quality of carrot and fruit juice wastes mixture through biological treatment (fermentation by using *Trichoderma viride*). The specific objectives were: to determine the effects of *Trichoderma viride* concentration and incubation period on the crude fiber, crude protein and ether extract of carrot and fruit juice wastes mixture and to obtain the proper concentration and incubation period for improving the quality of this juice wastes mixture. This experiment was designed in a 3 x 5 factorial experiment in a completely randomized design with 3 replication per treatment. The first factor was the concentration of *Trichoderma viride* (5, 7 and 9%) and the second factor was the incubation period (0, 5, 7, 9 and 11 days). In this experiment 100 g of carrot and fruit juice wastes mixture per experimental unit was treated with *Trichoderma viride* according to the concentration applied (5, 7 and 9%). There were 45 samples or experimental units in this experiment. All of the samples were fermented in solid state in the Laminar Flow for the period of 0, 5, 7, 9 and 11 days. After finishing the fermentation period, the *Trichoderma viride* in samples was killed by heating it at 80°C for 2 h in the oven. Then, samples were dried in oven at 60°C for 12 h for the preparation of the proximate analyses. Measured variables in this experiment were crude fiber, crude protein and ether extract content of carrot and fruit juice wastes mixture. Crude fiber, crude protein and ether extract were analyzed according to AOAC (1984). All of the data were statistically analyzed by analysis of variance of a factorial experiment in a completely randomized design. The difference among treatments was determined by DMRT procedure according to Steel and Torrie (1980).

Experiment 2: The purpose of this experiment was to compare the nutrient contents (crude protein, crude fiber, ether extract), fiber profile (NDF, ADF, cellulose, hemicellulose and lignin), amino acid profile, nitrogen retention and metabolizable energy between treated and non-treated carrot and fruit juice wastes mixture numerically. The treated sampel was obtained from the best of *Trichoderma viride* treated carrot and fruit juice wastes mixture in the experiment 1. Analyses of crude fiber, crude protein and ether extract were performed according to the proximate analysis procedures (AOAC, 1984), while NDF, ADF, cellulose, hemicellulose and lignin contents were determined according to Goering and Van Soest (1970). Amino acids contents of the carrot and fruit juice wastes mixture were performed by using HPLC. Nitrogen retention and metabolizable energy were assayed according to Sibbald (1986). All of the data obtained from untreated versus treated carrot and fruit juice wastes mixture were compared numerically.

RESULTS

Experiment 1

The effect of *Trichoderma viride* concentration and incubation period on crude fiber: The effect of *Trichoderma viride* concentration and incubation period on the crude fiber content of the carrot and fruit juice wastes mixture is shown in Table 2. The crude fiber content of carrot and fruit juice wastes mixture was very siginificantly affected ($p<0.01$) by the *Trichoderma viride* concentration. The incubation period also influenced this crude fiber very significantly ($p<0.01$). There was also a very significant interaction ($p<0.01$) between the *Trichoderma viride* concentration and the incubation period.

The effect of *Trichoderma viride* concentration and incubation period on crude protein: The effect of *Trichoderma viride* concentration (5, 7 and 9%) and incubation period (0, 5, 7, 9 and 11 days) on the crude protein content of carrot and fruit juice wastes mixture are depicted in Table 3.

The *Trichoderma viride* concentration very significantly affected the crude protein content of carrot and fruit juice wastes mixture. The incubation period also influenced

Table 2: The effect of *Trichoderma viride* concentration and Incubation period on crude fiber

Treatments	B0(0)	B1(5)	B2(7)	B3(9)	B4(11)	Averages
A1(5%)	15.10^{ABa}	15.21^{Aa}	13.97^{Cb}	14.34^{Bab}	15.34^{Aab}	14.79
A2(7%)	13.72^{Bb}	12.23^{Cb}	14.18^{Bb}	13.73^{Bb}	15.04^{Ab}	13.78
A3(9%)	13.26^{Cb}	14.58^{Ba}	15.42^{Aa}	14.55^{Ba}	15.90^{Aa}	14.74
Averages	14.03	14.03	14.52	14.21	15.43	

Table 3: The effect of *Trichoderma viride* concentration and Incubation period on crude protein

Treatments	B0(0)	B1(5)	B2(7)	B3(9)	B4(11)	Averages
A1(5%)	11.00^{Ba}	10.02^{Cb}	11.84^{Aa}	10.14^{Cc}	10.44^{Bc}	10.69
A2(7%)	11.00^{Ba}	11.29^{ABa}	10.05^{Cb}	11.60^{ABb}	11.94^{Ab}	11.18
A3(9%)	10.69^{Ca}	10.88^{Ca}	11.21^{Ca}	13.30^{Ba}	15.69^{Aa}	12.35
Averages	10.90	10.73	11.03	346.30	12.69	

Table 4: The effect of *Trichoderma viride* concentration and Incubation period on ether extract

Treatments	B0(0)	B1(5)	B2(7)	B3(9)	B4(11)	Averages
A1(5%)	4.25^{Ba}	4.41^{Ba}	4.31^{Bb}	5.37^{Aa}	4.39^{Bb}	4.55
A2(7%)	4.77^{ABa}	3.72^{Cb}	5.16^{Aa}	5.31^{Aa}	4.30^{BCb}	4.65
A3(9%)	4.52^{BCa}	4.24^{Cab}	4.89^{ABab}	4.85^{ABCa}	5.43^{Aa}	4.79
Averages	4.51	4.12	4.79	5.18	4.71	

Table 5: Nutrient content, nitrogen retention and metabolizable energy of untreated vs. Treated carrot and fruit juice wastes mixture

Compound and energy	Untreated	Treated biological
Crude fiber (%)	17.10	12.23
NDF (%)	34.30	31.55
ADF (%)	24.40	22.43
Cellulose (%)	12.20	11.15
Hemicellulose (%)	9.90	9.28
Lignin (%)	11.80	11.12
Crude protein (%)	8.40	11.29
Ether extract (%)	6.24	3.72
Nitrogen retention (%)	58.40	63.64
Metabolizable energy (kcal/kg)	1774.00	2557.00

very significantly ($p<0.01$) this crude protein content. There was a very significant interaction ($p<0.01$) between the *Trichoderma viride* concentration and the incubation period on this crude protein content.

The effect of *Trichoderma viride* concentration and Incubation period on ether extract: The effect *Trichoderma viride* concentration (5, 7 and 9%) and incubation period (0, 5, 7, 9 and 11 days) on the ether extract content of carrot and fruit juice wastes mixture is illustrated in Table 4.

The ether extract content of the carrot and fruit juice wastes mixture was not affected ($p>0.05$) by the *Trichoderma viride* concentration.

Experiment 2

The comparison of nutrient content, nitrogen retention and metabolizable energy between untreated vs. treated: The nutrient contents (crude fiber, crude protein and ether extract), fiber profile (NDF, ADF, cellulose, hemicellulose and lignin), nitrogen retention and metabolizable energy of carrot and fruit juice wastes is figured in Table 5.

Table 6: Amino acid profile of untreated vs. Treated carrot and fruit juice wastes mixture

Amino acids	Untreated	Treated
	------------------ (%DM)	------------------
Aspartate	0.71	0.60
Glutamate	0.90	0.78
Serine	0.32	0.34
Histidine	0.13	0.08
Glycine	0.41	0.41
Threonine	0.36	0.37
Arginine	0.31	0.26
Alanine	0.43	0.37
Tyrosine	0.23	0.25
Tryptophan	0.13	0.14
Methionine	0.06	0.10
Valine	0.41	0.41
Phenyl alanine	0.31	0.32
Iso-leucine	0.34	0.33
Leucine	0.48	0.48
Lysine	0.34	0.27
Cystine	0.01	0.03
Cysteine	0.02	0.02
Proline	0.50	0.51

The comparison of amino acid profile between untreated vs. treated carrot and fruit juice wastes mixture: The amino acid profile of the *Trichoderma viride* treated versus untreated carrot and fruit juice wastes mixture is shown in Table 6.

DISCUSSION

When the *Trichoderma viride* concentration was increased at the same incubation period, the crude fiber content declined. This result is in accordance with the result of experiment by Rizal *et al.* (2006a) in rutin-isolated cassava leaf, in which its crude fiber content was declined by fermentation using *Trichoderma viride* at the level of 6%. Iyayi and Aderolu (2004) also found a decrease in crude fiber content of brewer's dried grains, palm kernel meal, corn bran and rice bran when fermented with *Trichoderma viride*. When the incubation period was increased, the crude fiber content enhanced. The lowest crude fiber content was obtained at the *Trichoderma viride* concentration of 7% and incubation period of 5 days (A2B1). The increase in the *Trichoderma viride* concentration did not influence the crude protein content of the carrot and fruit juice wastes at the same incubation period. However, when the incubation period was augmented, there was a very significant increase in this crude protein content. The highest crude protein content was obtained from the *Trichoderma viride* concentration of 9% and the incubation period of 11 days (A3B4). The result of this experiment is different from the experiment by Ikhsan (1999), who found that the crude protein content of palm aren (*Arenga pinnata*) was enhanced by the fermentation with *Trichoderma viride* at the level of 7% for 9 days incubation period. The result of this experiment is also different from the result obtained by Rizal *et al*. (2006b), in which the dose of *Trichoderma viride* 7% was enough to increase the crude protein content of rutin-isolated cassava leaves. Iyayi and Aderolu (2004) also reported that the fermentation with *Trichoderma viride* enhanced the crude protein content of brewer's dried grains, palm kernel meal, corn bran and rice bran. The crude protein content of cassava peel was also increased by fermentation with *Trichoderma viride* (Ezekiel *et al*., 2010). However, the incubation period influenced this ether extract content. There was a very significant interaction between the *Trichoderma viride* concentration and the fermentation length on this ether extract content. The increase in the level of *Trichoderma viride* concentration did not influence the ether extract content of this juice wastes mixture at the same incubation period. However, when the incubation period was increased to a level of 5 days, there was a decline in the ether extract content of this juice waste. When the increase in this incubation period more than 5 days, the ether extract content of this juice wastes mixture augmented again. The lowest ether extract contain was reached at the *Trichoderma viride* concentration of 7% and the fermentation length of 5 days (A2B1). The result of this experiment is similar to the result of experiment by Rizal *et al*. (2006b), where the ether extract content of rutin-isolated cassava leaves reduced at 7% level of *Trichoderma viride*. The reduce in ether extract content of carrot and fruit juice wastes mixture was due to the degradation of this ether extract by lipase produced by *Trichoderma viride* (Wiseman, 1981; Rogers, 2002). When it is compared numerically, there is a decline in crude fiber, NDF, ADF, cellulose, hemicellulose, lignin and ether extract contents of *Trichoderma viride* treated carrot and fruit juice wastes mixture versus untreated one. On the other hand, the crude protein, nitrogen retention and metabolizable energy are enhanced by fermentation using *Trichoderma viride*. Rizal *et al*. (2006a) also reported that the fermentation of rutin-isolated cassava leaves with *Trichoderma viride* reduced their crude fiber, NDF, ADF, cellulose and hemicellulose contents. Meanwhile, Rizal *et al*. (2006b) found that the ether extract content of rutin-isolated cassava leaves was decreased by fermentation with *Trichoderma viride*. Iyayi and Aderolu (2004) reported that the energy levels of brewer's dried grains, palm kernel meal, corn bran and rice bran were enhanced by the fermentation with *Trichoderma viride*. The amino acid profile of carrot and fruit juice wastes mixture was influenced by *Trichoderma viride*. Some amino acids of the treated one declined, while the others were almost the same. The first limiting amino acid in the corn-soy poultry diet, ie., methionine slightly increased from 0.06 to 0.10%, while the second limiting amino acid lysine decreased from 0.34 to 0.27. The results of this experiment is different from those obtain by Ezekiel *et al*. (2010), in which the fermentation with *Trichoderma viride* increased the amino acid profile of cassava peel.

Conclusion: The fermentation by using 7% *Trichoderma viride* for 5 day incubation period was the best treatment for reducing crude fiber and improving the nutrient quality of juice wastes mixture. It is better than the untreated carrot and fruit juice wastes mixture nutrient content and nutrient quality.

ACKNOWLEDGEMENTS

This experiment was supported by the Foreign Research Cooperation and International Publication Funding Program provided by Directorate General of Higher Education, Ministry of National Education of the Republic of Indonesia. I am very grateful to the Dean of the Faculty of Animal Science and the Rector of the University of Andalas who have given me a chance to compete with others in obtaining this funding. My special gratitude is also directed to the Director General of Higher Education and the Minister of National Education who have provided this fundingss.

REFERENCES

Al-Betawi, N.A., 2005. Preliminary study on tomato pomace as unusual feed stuff in broiler diets. Pak. J. Nutr., 4: 57-63.

AOAC, 1984. Official Methods of Analysis (14th Edn.). Association of Official Analytical Chemists, Washington, DC.

Diarra, S.S., B.A. Usman and J.U. Igwebuike, 2010. Replacement value of boiled mango kernel meal for maize in broiler finisher diets. ARPN J. Agric. Bio. Sci., 5: 47-52.

Ezekiel, O.O., O.C. Aworh, H.P. Blaschek and T.C. Ezeji, 2010. Protein enrichment of cassava peel by submerged fermentation with *Trichoderma viride* (ATCC 36316). Afr. J. Biotech., 9: 187-194.

Ghazi, S. and A. Drakhshan, 2006. The effects of different levels of tomato pomace feedstuff on broiler performances. Department of Animal Science,College of Agriculture, Razi University, Iran.

Goering, H.K. and P.J. Van Soest, 1970. Forage Fiber Analyses. Agriculture Handbook No. 379, Agriculture Research Service, USDA, USA.

Ikhsan, I., 1999. The effect of innoculum *Trichoderma viride* levels an d fermentation period on crude protein content of palm aren wastes (*Arenga pinnata*). Sarjana S c rip tion. Faculty of Animal Science, University of Andalas, Padang.

Iyayi, E.A. and Z.A. Aderolu, 2004. Enhancement of the feeding value of some agro-industrial by-products for laying hens after their solid state fermentation with *Trichoderma viride*. Afr. J. Biotech., 3: 182-185.

NRC, 1994. Nutrient Requirements of Poultry. 9th Rev. Edn., National Academy Press. Washington DC.

Oluremi, O.I.A., V.O. Ojighen and E.H. Ejembi, 2006. The nutritive potentials of sweet orange (*Citrus sinensis)* rind in broiler production. Int. J. Poult. Sci., 5: 613-617.

Pelczar, M.J. and R.D. Reid, 1974. Microbiology. McGraw Hill Book Co., New York.

Rabayaa, E., J.M.A. Omar and R.A. Othman, 2001. Utilization of olive pulp in broiler rations. An-Najah University J. Res., Vol. 15.

Rizal, Y., Y. Harnentis, R. Marlida, Y. Sari and D.P. Sari, 2006a. Alteration of crude fiber, NDF, ADF, cellulose and hemicellulose contents of rutin-isolated cassava leaves under *Trichoderma viride* fermentation. J. Stigma, Vol. XIV, No. 1.

Rizal, Y., Y. Marlida, N. Farianti and D.P. Sari, 2006b. Effects of fermentation using *Trichoderma viride* on dry matter loss and organic matter, ash, crude protein, crude fat and HCN contents of rutin-isolated cassava leaves. J. Stigma, Vol. XIV, No. 1.

Rizal, Y. and G. Wu, 2009. Program of Academic Recharging Report. Project of Department of National Education, Republic of Indonesia.

Rizal, Y. and M.E. Mahata, 2009. Juice wastes prospect as alternative feed-stuffs for poultry. A Research Report. University of Andalas, Padang, Indonesia.

Rizal, Y., M.E. Mahata, M. Andriani and G. Wu, 2010. Utilization of juice wastes as corn replacement in the broiler diet. Int. J. Poult. Sci., 9: 886-889.

Rogers, J.M., 2002. Diamond V xp^{TM} DFM sets the standard in microbial feed technology. http://www.diamondv.com/newsrelease/xp_dfm_aug2002.html.

Sibbald, I.R., 1986. The TME System of Feed Evaluation: Methodology, feed composition data and bibliography. Animal Research Centre, Ottawa, Ontario.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures Statistics a Biometric Approach. McGraw Hill, New York.

Wiseman, A., 1981. Topics in Enzyme Fermentation Biotechnology. Vol. 4. Jhon Willey and Sons, New York.

Zafar, F., M. Idrees and Z. Ahmed, 2005. Use of apple by-products in poultry rations of broiler chicks in Karachi. Pak. J. Physiol., 1: 1-2.