

Thermal and probiotic treatment effects on restaurant waste for incorporation into poultry diet

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Abstract

Background Restaurant waste (RW) can be considered as a valuable feedstuff for animal nutrition due to its high nutritional value and low price. The aim of this study was to determine nutritional value and the microbial load of the waste in order to incorporate it in the poultry feedstuff. To our knowledge the current study is the first attempt in the field of RW processing with *Lactobacillus* strains. Then the second goal of this experiment was to study the effect of RW processing with *Lactobacillus* on nutrient and chemical composition of RW. The four *Lactobacillus* strains applied in the study included *Lactobacillus casei subsp-1608* ATCC 39392, *Lactobacillus plantarum-1058* ATCC 8014, *Lactobacillus acidophilus-1643* DSM 20079 and *Lactobacillus reuteri-1655* DSM 20016.

Results The experiments in the case of chemical composition showed that the mixed processing caused a significant improvement in nutritional value compared to the raw waste ($P < 0.05$). Among the strains, *L. acidophilus* had the highest mean amount of crude protein (CP), ether extract (EE), dry matter and gross energy (GE) with 21.40, 19.91, 41.02 and 4,872.61 kcal/kg, respectively, and the lowest amount of ash (3.67 %) compared with other strains. In the second phase of the experiment, the results obtained from comparing the average total count of bacteria and coliforms in five types of RW proved that the means of both bacteria

and coliforms are within the standard range and can be applied as poultry feed. However, regarding the average total count of bacteria, barbecue waste with 9.85×10^4 CFU/g and chicken waste with 9.51×10^3 CFU/g were the most contaminated and the healthiest foods, respectively. In addition, barbecue waste with 1.28×10^2 CFU/g and fish waste with 1.08×10^2 CFU/g had the highest and lowest count of coliforms, respectively.

Conclusion Nutritional value determination of RW samples showed that it is in the level that can be used in poultry diet. It was also presented that by adding *Lactobacillus* strains to the waste, the amount of GE, CP and EE increased and ash amount decreased. Therefore, its use in poultry diet is highly economic. In addition, this study showed that RW microbial load is in the level that can be applied as poultry feedstuff.

Keywords Probiotic · Nutritional value · Bacterial contamination · Restaurant waste · Processing method

Abbreviations

RW	Restaurant waste
CP	Crude protein
EE	Ether extract
DM	Dry matter
OM	Organic matter
GE	Gross energy
CF	Crude fiber
NFE	Nitrogen free extract
ME	Metabolizable energy

Introduction

Continuous rise of feed costs has forced livestock producers to find alternative choices for traditional feedstuffs.

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Restaurant waste (RW) is the leftover which can no longer be used in restaurants and hotels. Since it has high nutritional value and low price, RW can be considered as a valuable feedstuff for animal nutrition (Kojima 2005). Soliman et al. (1978) studied nutritional value of RW. They reported the values of 7.5, 21.6, 16.6, 4.6, 31.6, 18.3, 5.2 and 1.4 percent for moisture, crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen free extract (NFE), ash, calcium and phosphorus content of RW, respectively. Furthermore, Moradi (2012) calculated dry matter (DM), organic matter (OM), ash, CP, EE, CF and metabolizable energy (ME) of RW as following: 33.4, 95.9, 4.1, 15.1, 14.1, 12.3 percent and 3,270 kcal/kg, respectively. However, RW usage encountered some serious limitations because of its high potential to decay. Heat processing is one of the effective ways of reducing microbial contamination of feed. Sancho et al. (2004) suggested that food residual thermally processed at 65 °C for 20 min could be used as animal feed. However, due to high cost of heat processing, alternative methods can be considered.

Probiotics are microbial-biological sources which can be used for food processing (Kosin and Rakshit 2006). Probiotics usage alone or in mixed cultures can improve the nutritional value of the feedstuffs. They can also improve amino acid utilization through lowering protein degradation (Mikulec et al. 1999). Some *Lactobacillus* strains are capable of producing antimicrobial components when cultured on specific media (Jin et al. 1998). Avall-Jaaskelainen and Palva (2005) and Parvez et al. (2006) reported that Lactobacilli have antimicrobial effects and by producing some metabolites such as lactic acid, can restrict development of gram-positive and negative pathogens.

Rice grain is the main portion of meals served in Iranian restaurants; hence it comprises the highest proportion in the Iranian RW. During the cooking process, its moisture and fat content can be increased considerably. A supplementary foodstuff (called “Khoresht”) mainly consists of meat, peas, potatoes, tomatoes, onion and vegetables. However, depending on the type of Khoresht, the amount of nutrients, smell and flavor of RW can be variable.

Poultry's consuming RW has the risk of infection all the time because RW has high moisture and it must be used in a short period of time. In the divisions taken place according to food codex, the products containing meat and protein are among the high risk foods because the conditions required for bacteria to grow are present in them. According to the studies conducted all over the world, the accepted method to control the quality and hygiene of the cooked food is to use microbial standards. To assure the hygiene of the cooked food, Little et al. (2003) recommended some necessary experiments including the total count of bacteria, the count of coliforms especially *E. coli* and the count of pathogenic bacteria such as

Staphylococcus aureus, *Salmonella*, *Listeria monocytogenes* and *Clostridium perfringens*.

The aim of the present study was to determine nutritional value and microbial load of the waste in order to incorporate it in poultry feedstuff. To our knowledge, the current study is the first attempt in the field of RW processing with *Lactobacillus* strains. Then the second goal of this experiment was to study the effect of RW processing with *Lactobacillus* on nutrient and chemical composition of RW.

Methods

Sample preparation

The RW was obtained from the central restaurant of Tabriz university. In a period of 2 weeks, the fresh waste samples (78.7 % moisture) were collected daily and each meal sample was mixed with that of the other meals in an equal amount to keep the effect of each food. After gaining a homogeneous sample with an electric blender, the samples were divided into five parts (each part was 1 kg). One part as the control group (without any thermal processing) was dried in air conditions at room temperature and the other four experimental treatment groups were oven-dried at 60 °C for 20 min. Then the samples were immediately stored at -18 °C for later analysis (Westendorf et al. 1998). The laboratory studies were performed in the laboratories of faculty of veterinary medicine and faculty of agriculture of the University of Tabriz.

Lactobacillus culture preparation

Four *Lactobacillus* strains including *Lactobacillus casei* subsp-1608 ATCC 39392, *Lactobacillus plantarum*-1058 ATCC 8014, *Lactobacillus acidophilus*-1643 DSM 20079 and *Lactobacillus reuteri*-1655 DSM 20016 were used for microbial processing of RW. According to Jin et al. (1998) for each experiment, *Lactobacillus* strains were inoculated separately into Man, Rogosa, and Sharpe (MRS) broth and incubated at 37 °C for 24 h, after which the bacterial cells were harvested by centrifugation at 2,000×g for 10 min at 4 °C. To obtain a concentration of 1×10^8 cells/gram feed, the bacterial pellets were diluted with appropriate amounts of phosphate buffer solution (PBS), based on their original CFUs per gram determined on MRS agar. The freshly prepared bacterial suspensions were mixed into the feed each day to ensure viable bacterial cells in the feed throughout the experimental period. The viability of the bacterial cells was checked by MRS agar cultures to ensure that the concentration of the viable bacterial cells remained at 1×10^8 cells/gram feed.



Experimental groups and feed treatments

Experimental treatment groups included one negative control group (no process) and four heat and microbial treated groups. The samples were ground and blended together homogeneously for thermal and microbial processing. Then they were divided into four parts and kept into an oven for 20 min at 80 °C to remove the microbial contamination. Then, 5 ml of bacterial suspension of each strain was added to the RW samples and completely mixed in order to make uniform samples. The samples were kept at 37 °C of incubator for 24 h.

Chemical analysis

The CP, DM, ash and EE content of the samples were determined based on AOAC (1990) and the GE content was measured using *Labisco* Adiabatic bomb calorimeter.

Microbiological evaluation

To evaluate the microbial contamination of different kinds of RW (from distinct meals), the samples were collected during four continuous weeks and the average count of bacteria and coliforms were determined according to APHA and FDA procedures (Vanderzant and Splittstoesser 1992). The pour plate method was applied for calculation of total count of bacteria and the coliforms were enumerated by a violet red bile agar (VRBA) plate and brilliant green lactose bile broth (BGLB).

Data analysis

The statistical analysis was performed using the GLM procedure of SAS (2003) in one-way ANOVA design. Mean values were compared by Duncan multiple range test ($P < 0.05$) and Corr procedure was used to calculate the correlation coefficients.

Results

Chemical analysis

The average chemical composition of RW is reported in Table 1. Processing RW samples with *L. acidophilus* and *L. reuteri* increased the DM content compared to the control group ($P < 0.05$). The DM of the RW in the present study was 38.78 %. Also microbial processing of RW improved CP content in all treated groups compared to the control group; however the *L. acidophilus* and *L. reuteri* had the highest amount of CP content ($P < 0.05$).

Table 1 Chemical composition of restaurant waste in the experimental groups

	GE (kcal/kg)	CP (%)	DM (%)	EE (%)	Ash (%)
<i>L. acidophilus</i>	4,872.61 ^a	21.4 ^a	41.02 ^a	19.91 ^a	3.67 ^d
<i>L. casei</i>	4,625.47 ^c	19.51 ^b	38.67 ^c	19.2 ^b	3.90 ^c
<i>L. plantarum</i>	4,577.29 ^c	19.95 ^b	38.87 ^c	19.01 ^{bc}	4.02 ^b
<i>L. reuteri</i>	4,784.57 ^b	21.13 ^a	40.02 ^b	19.72 ^a	3.71 ^d
Control	4,507.33 ^d	18.87 ^c	38.78 ^c	18.84 ^c	4.12 ^a
Mean	4,673	20.17	39.47	19.34	3.88
SEM	19.52	0.18	0.16	0.07	0.03

Means within a column with no common superscript differ significantly ($P < 0.05$)

Data are from $n = 5$ replicate for each sample

Table 2 Correlation coefficient among chemical compositions of restaurant waste samples

	DM	Ash	CP	EE	GE
DM	1.00	-0.69	0.88	0.78	0.76
<i>P</i> value	-	0.0001	0.0001	0.0001	0.0001
Ash	-0.69	1.00	-0.83	-0.088	-0.095
<i>P</i> value	0.0001*	-	0.0001	0.0001	0.0001
CP	0.88	-0.83	1.00	0.87	0.89
<i>P</i> value	0.0001	0.0001	-	0.0001	0.0001
EE	0.78	-0.88	0.87	1.00	0.93
<i>P</i> value	0.0001	0.0001	0.0001	-	0.0001
GE	0.76	-0.95	0.89	0.93	1.00
<i>P</i> value	0.0001	0.0001	0.0001	0.0001	-

Replicate for each sample was 5

Asterisk indicates $P < 0.0001$

The results of this study showed that RW treated with *L. acidophilus*, *L. reuteri* and *L. casei* had higher fat than control group ($P < 0.05$), while in the *L. plantarum* treated RW and control group, the amount of fat was the same. The control group had the highest amount of ash content among experimental groups ($P < 0.05$), and the GE values of all treated samples were higher than those of the control group ($P < 0.05$; Table 1).

The correlation coefficients among chemical compositions of experimental groups are shown in Table 2. Crude ash had negative correlation with GE and CP ($P < 0.01$) and it also had high correlation between EE and GE ($r = 0.93$).

Microbial contamination

The results of evaluation in terms of microbial contamination revealed that the average total number of bacteria was in the normal range (10^5 CFU/g) and the highest and

Table 3 The average total count of bacteria (CFU/g) in five selected food waste of different types

	Mince	Chicken	Fish	Roasted chicken	Barbecue	Mean
1st w sample	6.42×10^2	1.90×10^4	2.59×10^4	5.31×10^2	1.27×10^5	$(3.46 \times 10^4)^a$
2nd w sample	1.36×10^5	5.24×10^2	3.84×10^2	2.56×10^2	4.30×10^3	$(3.33 \times 10^4)^a$
3rd w sample	3.15×10^4	1.62×10^4	2.28×10^3	3.78×10^3	1.22×10^5	$(3.51 \times 10^4)^a$
4th w sample	7.26×10^3	2.34×10^3	1.06×10^4	1.12×10^5	1.44×10^5	$(5.46 \times 10^4)^a$
Mean	$(4.38 \times 10^4)^{ab}$	$(9.51 \times 10^3)^b$	$(9.79 \times 10^3)^b$	$(3.54 \times 10^4)^{ab}$	$(9.85 \times 10^4)^a$	

Means within a row and column with no common superscript differ significantly ($P < 0.05$)

Table 4 The average count of coliforms (CFU/g) in five selected food waste of different types

	Mince	Chicken	Fish	Roasted chicken	Barbecue	Mean
1st w sample	1.23×10^2	1.27×10^2	1.33×10^2	1.19×10^2	1.2×10^2	$(1.24 \times 10^2)^a$
2nd w sample	1.21×10^2	1.13×10^2	9.4×10^1	1.42×10^2	9.3×10^1	$(1.12 \times 10^2)^a$
3rd w sample	9.7×10^1	9.8×10^1	9.1×10^1	8.8×10^1	1.67×10^2	$(1.08 \times 10^2)^a$
4th w sample	1.26×10^2	10^2	1.15×10^2	9.6×10^1	1.32×10^2	$(1.13 \times 10^2)^a$
Mean	$(1.16 \times 10^2)^a$	$(1.09 \times 10^2)^a$	$(1.08 \times 10^2)^a$	$(1.11 \times 10^2)^a$	$(1.28 \times 10^2)^a$	

Means within a row and column with no common superscript differ significantly ($P < 0.05$)

lowest number were for barbecue (Kebab) waste and chicken waste, respectively ($P < 0.05$; Table 3). The average counts of coliforms was a little higher than standard, (10^2 CFU/gram). However, no significant differences were observed among experimental groups (Table 4).

Discussion

The DM of the RW in the present study (38.78 %) was higher than reports of Walker et al. (2002) with 37.46 %, Kjos et al. (2000) with 21.4 % and Moradi (2012) with 33.4 %. The CP content of RW was similar to the results of Saki et al. (2006); however, it was higher than the results of Moradi (2012) and Kojima (2005) with 15.1, 15.6 %, respectively. The other researchers (Walker et al. 1998, 2002, 2004; Kjos et al. 2000; Westendorf et al. 1998) reported higher amount of CP with 33.4, 29, 20, 49 and 21.4 %, respectively.

The variation in chemical composition of RW in different studies could be due to the use of different ingredients in preparing food, additives and cooking methods. In Iran, rice comprising the main part of food in restaurants. Therefore, the major part of RW is rice with leftover vegetables, cereals and stew.

Processing RW with Lactobacilli causes the production of some bacterial metabolites during proliferation like *Bacteriocins* as proteinaceous toxins (Ogunbanwo et al. 2003). *Lactobacillus* strains excrete several extra-cellular enzymes which could digest the nutrients such as RW.

Then, the existing microorganisms on the surface of RW can use these materials for their growth and proliferation.

Increasing the microbial mass during the processing can increase the CP content in the final product. Also some part of higher CP content in microbial processed RW can be due to the production of *Bacteriocins* during the proliferation period. Kailasapathy (2002) and Angel et al. (2005) suggested that, microorganisms could use structural carbohydrates of food as a source of energy and using nitrogenous compound can produce their own needed protein. So, microbial processing of RW can enrich it by biological conversion method. Moreover, most of the *Lactobacillus* strains contain a S-layer composed of protein and glycoprotein units. S-layer is known as a protective stratum which preserves the cell shape and traps the ions and molecules in the ectoblast of membrane of bacteria. As *Lactobacillus* grows in the processed medium, the number of S-layer gets increased. It also increases the protein content of the processed food (Frece et al. 2005).

Frece et al. (2005) demonstrated that *L. acidophilus* has more proteolytic activity compared with the other Lactobacilli. Due to this feature, it can produce more nitrogenous compounds which are necessary ingredients for bacterial growth. Availability of free nitrogen through proteolysis can enhance bacterial population by producing more *Bacteriocin* and increasing the number of S-layer. These phenomena can increase the protein content of the processed feed. Therefore, the higher CP content of RW which was processed by *L. acidophilus* in the present study can be a result of this function (Table 1).



Kafilzadeh and Safariparvar (2003) reported that the positive effects of feed supplementation with *Lactobacillus* spp. could be due to several factors including: supplying some nutrients such as vitamins, producing some digestive enzymes, destroying pathogenic bacteria and neutralizing their toxins by producing organic acids and *Bacteriosins*. Zhang et al. (2003) suggested that *Lactobacillus* could improve nitrogen retention and nutrient absorption especially fatty acids and glucose, therefore they could be considered as a growth promoter for improvement of the livestock performance.

EE or fat content of RW in the present study (18.84 %) was less than some reports (Westendorf et al. 1998 with 27.2 % and Kjos et al. 2000 with 33 %). However, it was higher than the other reports (Walker et al. 2002; Saki et al. 2006; Moradi 2012 with 15.4, 10.58 and 15.1 %, respectively). The foodstuff, supplied oil and cooking method can influence the amount of fat in the RW.

The difference in the amount of fat among *Lactobacillus* strains primarily could be due to their different growth behavior in different conditions. Bagley et al. (2005) suggested that, *Lactobacillus* spp. bacteria grew conveniently in MRS- Sorbitol- Agar and MRS- Salicin- Agar cultures in laboratory conditions and create clear and large colonies by fermentation of the existing carbohydrates. However, when the same strains are cultured in different mediums, their purity and efficacy could be different. Regarding the fact that membrane of cellular organelles and the whole membrane systems existing in the cells are made of Lipoproteins (two layers of phospholipid and a layer of protein), by increasing bacterial mass during the processing, the amount of phospholipids increases as well. Probably, more growing strains could have more cellular mass and fat content.

Different bacterial strains have different ability in destroying pathogenic strains. *L. acidophilus* and *L. reuteri* produce *Lactacins* and *Reuterin* (a β -3-HPA compound derived from glycerol), respectively, which have a vast functional effect against gram negative and positive bacteria and can destroy the pathogenic bacteria more effectively than the other strains (Casas Ivan and Dobrogosz Walter 2000; Parvez et al. 2006). Therefore, the higher amount of fat and other nutrients in these two groups is not so much surprising. Kailasapathy (2002) demonstrated that when *Lactobacillus* is injected to a sterile food, the immunity challenge to overcome the pathogenic strains does not happen, therefore, the energy requirements decrease. In this case, the extra accessible energy could shift for fat synthesis.

Samarasinghe et al. (2003) reported increased fat content with supplemented *Virginiamycin*, while Sabatkova et al. (2008) observed no difference in the amount of EE in diets containing antibiotic and incorporated with three

strains of probiotic. The variation in different reports could be due to the composition of substrates and the amount of fat existing in food.

Saki et al. (2006) reported lower GE than our results (4,300 vs. 4,507 kcal/kg). The higher amount of GE in all of the processed treatments comparing the control group reflects the higher content of nutrients in these groups which originated from the proliferation of *Lactobacillus* strains. Avall-Jaaskelainen and Palva (2005) reported that organic acid produced by *Lactobacillus* can probably prevent the growth of pathogenic microorganisms and increase energy content in the processed food. Also, Misstotten et al. (2007) reported increased amount of lactic acid and exoenzymes production after the addition of probiotic to carrot waste.

According to the results of present study, higher amounts of CP and EE in experimental treatments, can increase GE content, however, it should be noticed that the GE is not a good criterion for accessible energy of the feedstuffs by the animal (Walker et al. 2004).

Saki et al. (2006) reported higher amount of ash compared to the current study (6.29 vs. 4.12 %). Higher amount of ash resulted in lower value of organic matter and subsequently decrease in the content of GE. Some researchers previously reported negative correlation between the ash with CP and EE content (Westendorf et al. 1998; Walker et al. 2002). Considering the high correlation between EE and GE ($r = 0.93$), the main reason for difference among GE of experimental treatments could be their different amounts of EE. Therefore, the higher amount of GE in all of the processed treatments can be explained by higher bacterial population and consequently increased amount of EE and CP content of them (Table 2).

The results of evaluation in terms of microbial contamination showed the highest total count was for barbecue (Kebab) waste. Improper heating during the cooking process may be the main reason for higher bacterial counts in the kebab. Cooking method for kebab is direct heating for a short time, and there is not enough time for removal of the contaminant bacteria. Tessi et al. (2002) mentioned that longer heating time can provide more safety margin for food.

This experiment revealed that microbial contamination of RW is in an acceptable range for use as poultry feed. Probably, a simple and economic processing of RW (i.e. thermal processing) can overcome the anxiety about probable infection.

Tessi et al. (2002) studied 101 samples of cooked and ready to serve food in a central school kitchen in Argentina and found that the average of bacterial and coliform contamination were 3.63×10^4 and 1.90×10^2 /g, respectively. He separated 6.34 % *E. coli* from the samples. Furthermore, Fang et al. (2003) studied 164 samples of

cooked food waste in Taiwan. He reported contamination rates 27.5, 17.9, 7.9 and 4.98 % for coliforms, *Staphylococcus aureus*, *E. coli* and *Bacillus cereus*, respectively.

Fang et al. (2003) reported that favorite thermal range for growth of *Salmonella*, bacteria and fungi is 5–55 °C. Raising temperature to 60 °C, caused the microorganisms to stop their development, however, microbial mass reduction follows a logarithmic pattern. Bacterial death rate depends on pH level as well as inner moisture. Proper pH for growth of *Salmonella* and bacteria is 7–8.5 and for fungi is 5–7. Supplementation of feed with different probiotic strains, can decrease microbial contamination through pH level.

Lactobacillus casei and *L. plantarum* strains are commonly used in feed manufacturing to improve feed quality and compete with decaying bacteria of the food and sometimes with pathogenic bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes* (Ogunbanwo et al. 2003). Patterson and Burkholder (2003) indicated that coliform and clostridia had a negative influence on intestinal mucosa and nutrient absorption in the broilers. Therefore, it is suggested that these probiotics be used as a feed additive to RW.

Low dry matter content of RW makes it susceptible to microbial decay which is the main challenge in consuming these kinds of feed. However, Rameshi et al. (2007) reported that wet feed usage in broiler chicken rations, increased weight gain and improved feed efficiency. Considering high moist content of RW, the processing of feed containing RW with probiotics, not only prevents feed decay, but also improves broiler performance by increased digestibility and nitrogen retention.

Conclusion

The nutritional value determination of RW samples showed that it is in the level applicable in poultry diet and by adding *Lactobacillus* strains to the waste, GE, CP and EE increased and ash amount decreased. Among the *Lactobacillus* strains, *L. acidophilus* was the most efficient. The difference among the resulted contents for *Lactobacilli* strains in the current study is due to their different capabilities in producing different metabolites which are able to increase the nutritional value of the feed. Since there is not enough data in the case of RW microbial processing, it is suggested that other studies be carried out in this field to make RW use more applicable as poultry feed. It is also recommended that the effect of other microbial strains be investigated in order to find a strain which has the most efficient performance on RW. In addition, this study showed that RW microbial load is in the level that can be applied as poultry diet. Furthermore, one of the most

important advantages of using *Lactobacillus* in RW is to turn the food which is fed to the broilers and consequently the food which human beings consume into healthy food. This privilege is more significant than its effect on poultry productive performance. Therefore, its use in poultry diet is highly economic.

Conflict of interest The authors declare that they have no competing interests.

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