http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2018.110.118

Research Article
Effect of Partial Substitution of Ration's Soybean Meal by Biologically Treated Feathers on Rumen Fermentation Characteristics (*in vitro*)

[1]Siham A. Ismail, [1]Azza M. Abdel-Fattah, [1]Mohamed A. Emran, [2]Hossam H. Azzaz, [3]Mamdouh S. El-Gamal and [1]Amal M. Hashem

[1]Department of Chemistry of Natural and Microbial Products, Division of Pharmaceutical and Drug Industries Research, National Research Centre, Giza, Egypt
[2]Department of Dairy Sciences, National Research Centre, 33 Buhouth St, Giza, Egypt
[3]Department of Botany and Microbiology, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt

Abstract

Background and Objective: Feather wastes are the most abundant keratinous material in the nature and its accumulation causes multiple environmental problems. Nutritive value upgrading of such wastes through biological treatments may provide ruminant's rations with high quality and cost effective source of protein. Therefore, the main objective of this study was to investigate the potential uses of biologically treated feathers (BTF) as a feedstuff for ruminants through *in vitro* experiments. **Materials and Methods:** Keratinase production time course was performed by ten microbial isolates (3 fungal, 3 actinomyces and 4 bacterial isolates) under static and shaking conditions using turkey feather- synthetic medium. The chemical composition and amino acid analysis for the crude feathers, BTF and soybean meal were determined according to AOAC methods. Two *in vitro* experiments were conducted to study the effects of crude feathers, BTF and modified ruminant rations (in which soybean meal were substituted by the BTF in 10, 20 and 30%) on rumen fermentation characteristics. Ration's Dry Matter (DM), Organic Matter (OM), Neutral detergent fibre (NDF) and Acid detergent Fibre (ADF) degradability by rumen microorganisms were tested using batch culture technique. Ruminal final pH, ammonia-nitrogen, total volatile fatty acids and short chain fatty acids concentrations were determined after 24 h of incubation. The total gas production volume was determined using 100 mL glass syringes. **Results:** *Bacillus licheniformis* ALW1 was the most potent keratinase producer strain under static condition at 37°C for four days of incubation. Feather biological treatment by *Bacillus licheniformis* increased its content of some of essential-sulphur amino acids. The degradability of BTF by rumen microorganisms was 4 folds higher than crude feather degradability. There were no significant differences between control and partially substituted (R_{10} and R_{20}) rations in all of rumen fermentation characteristics. **Conclusion:** The utilization of BTF as substitute for costly soybean meal in ruminant's rations up to 20% had no negative effect on all rumen fermentation characteristics.

Key words: Biologically treated feathers, *Bacillus licheniformis*, rumen fermentation characteristics, *in vitro*, soybean meal and ruminant rations

Citation: Siham A. Ismail, Azza M. Abdel-Fattah, Mohamed A. Emran, Hossam H. Azzaz, Mamdouh S. El-Gamal and Amal M. Hashem, 2018. Effect of partial substitution of ration's soybean meal by biologically treated feathers on rumen fermentation characteristics (*in vitro*). Pak. J. Biol. Sci., 21: 110-118.

Corresponding Author: Hossam H. Azzaz, Department of Dairy Sciences, National Research Centre, 33 Buhouth St, Giza, Egypt
Tel: 202 33068626 Fax: 202 33370931

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry feather is the most abundant keratinous material in the nature as β-keratin represents around 90% of feather weight[1]. Annually, poultry processing industry generates a huge amount of feathers as a waste. As feather wastes had a serious impact on the environment, it was necessary to manage its accumulation by economically safe manner[2,3]. The utilization of such wastes in the ruminant feeding may give alternative solution to overcome their environmental problems and to provide ruminant's diets with cheap protein source. Unfortunately, it has been reported that feather protein is poorly digested in birds and mammals[4]. This poor digestion of keratin molecules have been attributed to disulfide bonds which are hard to degrade by animal's proteolytic enzymes[5]. Also, lack of feather protein in essential amino acids decreases its biological and nutritive value. These problems could be handled through hydrolysis of feathers by thermal or chemical treatments. The utilization of thermal or chemical hydrolyzed feathers in feeding of cattle, sheep and dairy goats has been reported[6-9]. It's appearing from these studies that overall nutritive properties of feathers protein are affected by processing conditions. In this concern, the thermal treatment of feathers required high in put and costly energy and the resulted feather meal suffered from low digestibility and variable nutrient bioavailability[10]. The formation of amino acids with non-nutritive character is another reason for switching to an alternative technology[1,11].

Improvement of feather digestibility and amino acid balance could be achieved by the biological treatments. The ability of different microorganisms for poultry feathers hydrolysis has been reported[12,13]. Fermented feather meal production by non-pathogenic microbes may provide diets of ruminants with high quality and cost effective source of protein. So, the main aim of this study was to investigate effects of partial substitution of ration's soybean meal by biologically treated feathers (BTF) on rumen fermentation characteristics (*in vitro*).

MATERIALS AND METHODS

Substrate: Turkey feathers were collected from Kafr Ghataty, Al-Haram, Giza, Egypt and were soaked for 12 h in a washing liquid containing 1% detergent, then washed thoroughly with distilled water. The feathers were then dried completely at 60°C, milled and used as substrate for microbial keratinase production.

Microorganisms and inoculum preparation: Ten of microbial isolates (3 fungal, 3 actinomycetes and 4 bacterial isolates) from feather, leather and wool samples were screened for their ability for keratinase production. The fungal isolates were maintained on slants of potato dextrose agar (PDA) medium, while the actinomyces and bacterial isolates were maintained on slants of tryptone soya agar (TSA) medium. Synthetic medium composed of (g L^{-1}): glucose 10.0; peptone 10.0; yeast extract 3.0; $CaCl_2.2H_2O$ 2.0; pH 7.0±0.2 was used for preparing the activated fungal, actinomyces and bacterial inocula. As described by Abdel-Fattah *et al.*[14], ten sterilized milliliter of the previously mentioned medium were added to each slant of the tested isolates and scratched with a sterile needle. This suspension was transferred to 250 mL erlenmeyer flasks containing 40 mL of the same medium. These flasks were incubated in shaking incubator (Thermo Scientific MaxQ481RHP) at 180 rpm for 72h at 30°C for fungi cultivation and for 48 h at 37°C for cultivation of bacteria and actinomycetes.

Production medium: For screening of the most effective keratinase producing isolate, 250 mL Erlenmeyer flasks contain 50 mL growth medium were used. The growth medium has the following composition (g L^{-1}): NaCl 0.5; KH_2PO_4 0.7; K_2HPO_4 1.4; $MgSO_4.7H_2O$ 0.1; 2% (w/v) of prepared feather in pH 7.0±0.2[15]. Three flasks were separately inoculated with 5% inoculum size of each tested isolate and incubated under static and shaking (180 rpm) conditions for different incubation periods. At the end of the fermentation periods the keratinase activity was measured after centrifugation of the fermented medium at 5000×g for 15 min.

Keratinase assay: The keratinolytic activity of culture filtrate was assayed according to the method of Cai *et al.*[16] using soluble keratin as substrate. Where, one unit of keratinolytic activity was defined as an amount of enzyme which cause increase in absorbance at 280 nm of 0.01 min under standard reaction condition.

Preparation of biologically treated feather (BTF): The BTF was produced by fermentation of feather using the most potent keratinase producer microorganism. At the end of the fermentation period, the fermented feather was sieved through 2 mm pore sieve to remove coarse feathers and then dried completely by lyophilization for further *in vitro* application.

Table 1: Amino acid profile of crude feather, biologically treated feathers and soybean meal

Amino acid	Weight per crude protein (%)		
	Crude feather	Biologically treated feathers (BTF)	Soybean meal
Aspartic (ASP)	6.13	6.24	11.53
Threonine (THR)	3.47	3.45	3.41
Serine (SER)	8.90	7.38	5.24
Glutamic (GLU)	8.26	8.72	16.38
Glycine (GLY)	7.74	5.71	4.26
Alanine (ALA)	6.18	4.57	4.36
Valine (VAL)	7.26	5.31	4.28
Isoleucine (ILE)	3.35	3.68	4.15
Leucine (LEU)	7.33	5.42	7.33
Tyrosine (TYR)	3.87	3.50	3.46
Phenylalanine (PHE)	5.05	5.93	4.98
Histidine (HIS)	0.56	1.23	2.50
Lysine (LYS)	0.91	2.38	5.96
Arginine (ARG)	5.67	4.90	6.99
Proline (PRO)	8.90	7.87	5.03
Cystine (CYS)	6.69	6.02	1.06
Methionine	0.29	0.62	1.06
Total	90.56	82.92	91.98

Table 2: Chemical composition and *in vitro* degradability (%) of soybean meal, crude feather and the biologically treated feathers (on DM basis)

Item	Soybean meal	Crude feather	BTF	SE±
DM	88.88	91.74	93.22	8.05
OM	93.27	99.61	78.37	39.81
NDF	15.06	2.69	0.89	29.23
ADF	6.46	2.41	0.67	11.00
CP	38.76	93.30	55.15	1.02
EE	4.78	0.73	1.58	7.79
Ash	6.73	0.40	21.63	39.79
NFC	34.67	2.88	20.75	58.19
IVDMD (%)	68.64^{a}	13.71^{c}	51.68^{b}	10.30
IVOMD (%)	73.39^{a}	14.46^{c}	62.02^{b}	11.45

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, NFC: Non fiber carbohydrate, BTF: Biologically treated feathers, IVDMD (%): *In vitro* dry matter degradability and IVOMD (%): *In vitro* organic matter degradability, $^{a-c}$Means at the same row with different superscript are significantly ($p<0.05$) different, ±SE: Standard error

Amino acid measurement: Quantitative amino acid measurements were performed for the crude feather, biologically treated feathers and soybean meal (Table 1) according to official method of analysis No. 994.12[17]. The system used for analysis was high performance amino acid analyzer (Biochrom 30) with EZChrom Software for data collection and processing.

Feed ingredients chemical composition: Feed ingredients (including crude feathers and BTF) were ground through 2 mm screen on a Wiley mill grinder and then analyzed according to the AOAC methods[18] to determine dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash contents. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using the methods described by Van Soest *et al.*[19].

***In vitro* study:** Two *in vitro* experiments were conducted for investigating the potential use of BTF as ruminant feed ingredient. In the first one, ruminal "*in vitro*" dry matter and organic matter digestion (IVDMD and IVOMD) for soybean meal, BTF and crude turkey feathers (substrates) have been determined as described by Tilley and Terry[20]. The chemical composition and *in vitro* Dry Matter Degradability (IVDMD %) and *in vitro* Organic Matter Degradability (IVOMD %) of the experimental substrates shown in Table 2.

In the second, batch fermentation culture experiment was conducted according to El-Sherbiny *et al.*[21] to evaluate the effect of partial substitution of ration's soybean meal by biologically treated feathers on rumen fermentation characteristics. A total mixed ration consisted of 30% berseem (clover) hay, 25% yellow corn, 20% corn stalks, 12% soybean meal, 12% wheat bran and 1% minerals and vitamins mixture was used as a substrate. For obtaining of the rumen microorganisms (inoculum), rumen fluid was collected from rumen of slaughtered rams fed berseem hay ration. The powdered BTF was substitute the soybean meal of total mixed ration at the following levels: 0, 10, 20 and 30% on DM basis. The feed ingredients and the chemical composition of the experimental rations were shown in Table 3. Each treatment was tested in 3 replicates accompanied by 3 blank vessels (no substrate). The tested rations (400 mg) were added separately to the 125 mL incubation vessels. Each vessel was filled with 40 mL of mixture of 1:3 (v/v) rumen fluids: buffer solution. All vessels were sealed and incubated at 39°C for 24 h. After 24 h of incubation, all vessels were filtered in fiber filter bags 25 micron porosity (ANKOM- USA). The residues in the bags were dried at 70°C in oven for 48 h to analyse dry matter (DM), organic matter (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility. Rumen fluid pH was measured using (pH-meter). Overall volume of the produced gases was determined using Hohenheim Syringes (100 mL) as described by Navarro-Villa *et al.*[22]. Quantitative analysis of ammonia concentration was carried out by a modified Nessler's method[23]. The total volatile fatty acids (VFA) were determined by steam distillation method as described by Warner[24]. The short chain fatty acids (SCFA) concentration was calculated according to Eq. of Makkar[25]:

$$SCFA\ (mmol) = 0.0222\ Gas - 0.00425$$

Where:
Gas = Gas production at 24 h incubation (mL/200 mg DM)

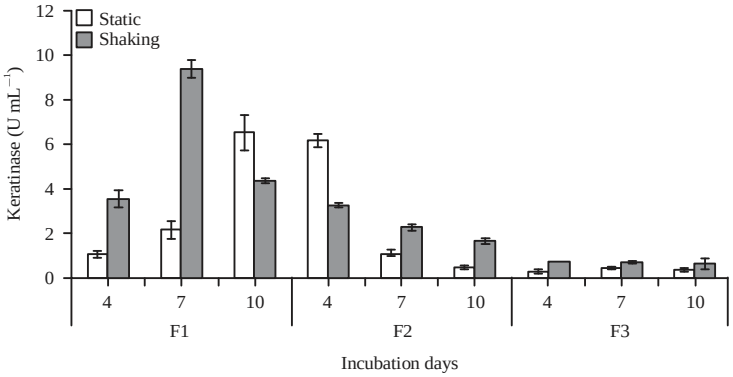

Fig. 1: Time course production of keratinase by fungal isolates

Table 3: Chemical composition of feed ingredients and the tested rations (on DM basis)

	DM (g kg^{-1})							
Items	DM	OM	NDF	ADF	CP	EE	Ash	NFC
Feed ingredients								
Corn grain	884.50	985.50	184.40	35.90	82.50	53.15	14.50	665.45
Soybean meal	888.80	932.70	150.60	64.60	387.60	47.80	67.30	346.70
Wheat bran	893.30	956.00	352.10	98.30	152.60	37.60	44.00	413.70
Corn stalks	926.00	941.00	700.00	430.00	30.00	15.00	59.00	196.00
Clover hay	924.00	867.90	409.40	268.80	174.10	39.80	132.10	244.60
BTF	932.20	783.70	8.87	6.68	551.50	15.80	216.30	207.53
Experimental rations								
Control	897.38	921.59	369.24	195.16	143.68	38.48	68.41	370.19
R_{10}	897.90	919.80	367.54	194.44	145.65	38.09	70.20	368.52
R_{20}	898.42	918.01	365.84	193.77	147.61	37.71	71.99	366.85
R_{30}	898.94	916.23	364.14	193.08	149.58	37.32	73.78	365.18

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, NFC: Non fiber carbohydrate and BTF: Biologically treated feathers, Control: Concentrate feed mixture (Corn grain 25%, Soybean meal 12%, Wheat bran 12%, 1 minerals mixture)+Corn stalks 20%+Clover hay 30%, R1: Concentrate feed mixture (Corn grain 25%, Soybean meal 10.8%, Wheat bran 12, 1.2% BTF, 1% minerals mixture)+Corn stalks 20%+Clover hay 30%, R2: Concentrate feed mixture (Corn grain 25%, Soybean meal 9.6%, Wheat bran 12%, BTF 2.4, 1% minerals mixture)+Corn stalks 20%+Clover hay 30%, R3: Concentrate feed mixture (Corn grain 25%, Soybean meal 8.4%, Wheat bran 12, 3.6% BTF, 1% minerals mixture)+Corn stalks 20%+Clover hay 30%

Statistical analysis: Data obtained from this study were statistically analysed by IBM SPSS Statistics for Windows[26] using the following general model procedure:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = Parameter under analysis of the ij bottles of rumen liquor trails

μ = Overall mean

T_i = Effect due to treatment on the parameter under analysis

e_{ij} = Experimental error for ij on the observation

Duncan's multiple range tests was used to test the significance among means[27].

RESULTS

Screening for keratinase production: Isolation and identification of new strains able to degrade insoluble keratinous materials to valuable products is one of the aims of this work. Keratinase production by fungal isolates (Fig. 1) indicated that isolate F1 gave the best productivity (9.39 U mL^{-1}) after 7 days of incubation under shaking condition, while the productivity was reduced to 4.35 U mL^{-1} after ten days of incubation in F1. Under static condition, the highest keratinase productivity (6.54 U mL^{-1}) was observed by fungal isolates (F1) after ten days of incubation.

Isolates belong to actinomyces were screened for keratinase biosynthesis and the results in Fig. 2 showed that isolate A1 gave the best yield (13.81 U mL^{-1}) after 7 days of incubation under shaking condition. However, the highest

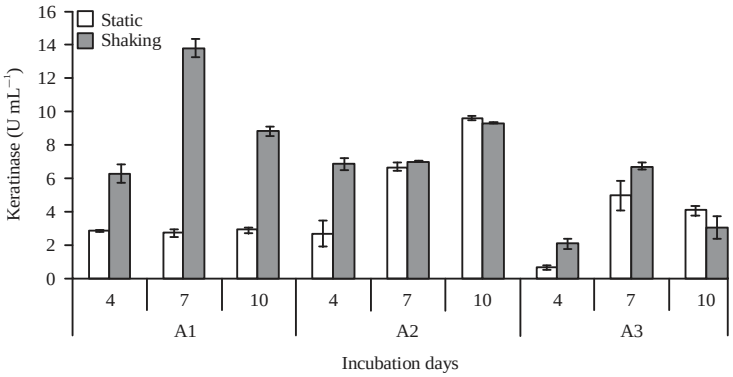

Fig. 2: Time course production of keratinase by actinomyces isolates

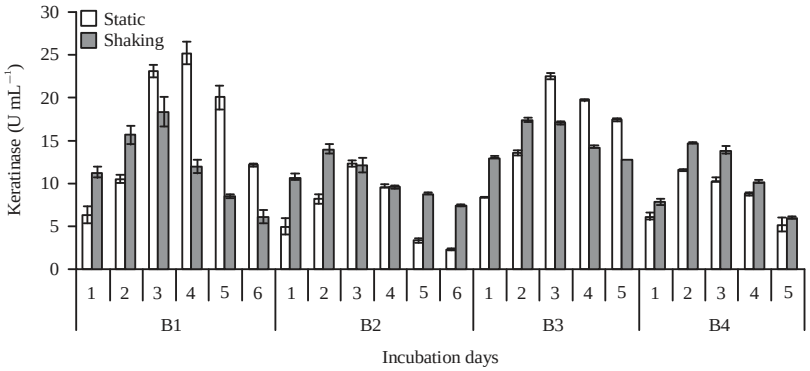

Fig. 3: Time course production of keratinase by bacterial isolates

keratinase productivity under static condition (9.62 U mL^{-1}) was observed by isolate A2 after ten days of incubation.

The production of keratinase by bacterial isolates under static and shaking conditions shown in Fig. 3. The maximum keratinase production (25.17 U mL^{-1}) was observed under static condition by isolate B1 after 4 days of incubation followed by isolate B3 (22.5 U mL^{-1}) after 3 days of incubation. The highest keratinase production under shaking conditions (18.36 U mL^{-1}) was observed after 3 days of incubation for isolate B1.

Among all fungal, actinomyces and bacterial isolates investigated for keratinase production the bacterial isolate B1 was the most promising keratinase producer. The results of 16S rDNA gene sequence and transmission electron microscopy (data not shown) indicated that B1 was *Bacillus licheniformis* isolate. The data was submitted to Gen Bank under the name of *Bacillus licheniformis* ALW1 with accession no. LC315920.

Based on these results, *Bacillus licheniformis* ALW1was used for the biological treatment of the feathers and subsequent experiments related to the effect of the biologically treated feathers on the rumen fermentation characteristics (*in vitro*).

Amino acids analysis: The amino acids composition of crude feather (untreated feather), biologically treated feathers (BTF) and soybean meal were presented in Table 1. The profile of the amino acids of BTF was similar to that of crude feather; however BTF shows higher percentage of essential amino acids histidine, lysine, methionine and phenylalanine. Moreover, it's obvious that soybean meal is superior over BTF and crude feather in aspartic, glutamic, isoleucine, histidine, lysine, arginine and methionine amino acids percentages expressed as weight per crude protein. In contrast, soybean meal has deficiency in cystine, proline, phenylalanine, tyrosine, valine, alanine, glycine, serine and threonine amino acids when compared with BTF and crude feather.

Chemical composition of feed ingredients: The chemical composition of crude feather was different from that of BTF and soybean meal (Table 2). The crude protein content of BTF (55%) was less than crude feather (93%), while soybean meal recorded the lowest protein content with around 39% (on dry matter basis). In contrast, ether extract (EE) of the crude feather recorded the lowest percentage (0.7%) followed by BTF (1.5%), while soybean meal recorded the highest

Table 4: Effect of tested rations on *in vitro* rumen fermentation characteristics

Items	Control	R_{10}	R_{20}	R_{30}	SE±
Degradability (%)					
Dry matter	57.85	56.12	56.33	55.95	0.37
Organic matter	63.69[a]	62.06[ab]	62.14[ab]	61.59[b]	0.35
Neutral detergent fiber	37.40	34.21	37.56	37.18	0.68
Acid detergent fiber	19.13[a]	16.16[ab]	15.35[ab]	13.87[b]	0.85
Rumen basic parameters					
Total gas production (mL/24 h)	137.50[a]	135.50[ab]	134.00[ab]	133.50[b]	0.69
pH	6.67	6.70	6.69	6.73	0.01
Ammonia-nitrogen (μmol L^{-1})	2.56	2.50	2.61	2.58	0.10
Total volatile fatty acids (mEq dL^{-1})	7.10	6.95	6.85	6.80	0.12
Short chain fatty acids (mmol)	1.70[a]	1.66[ab]	1.65[ab]	1.64[b]	0.01

[a-b]Means at the same row with different superscript are significantly ($p<0.05$) different, ±SE: Standard error

percentage of EE with 4.8%. It was obvious that BTF had the highest percentage of ash (22%) and consequently it has the lowest percentage of organic matter (78%) followed by soybean meal, while crude feather shows the highest and the lowest values of organic matter (99%) and ash (0.4%) respectively. Moreover, soybean meal had the highest percentages of NDF (15.06%), ADF (6.46%) and NFC (34.67%), while the BTF had the lowest values of NDF (0.9%) and ADF (0.7%). The NFC level of BTF (21%) was seven-fold greater than crude feather (3%). Inclusion of BTF in ruminant rations as a replacer for soybean meal at different levels (0, 10, 20 and 30%) has been investigated in the current study. Ration's chemical proximate analysis showed slight gradual increase in the experimental ration's DM; CP and ash contents with increasing BTF inclusion level (Table 2). In contrast, slight gradual decrease in the experimental ration's OM, NDF, ADF, EE and NFC contents was occurred with increasing level of BTF inclusion.

***In vitro* experiments:** Results of *in vitro* dry matter degradability (IVDMD %) and *in vitro* organic matter degradability (IVOMD %) Table 2 showed that crude feathers gave the lowest ($p<0.05$) IVDMD % and IVOMD % by rumen microorgansimes. The biological tretment for the crude feathers improve its IVDMD % from 13.71 to 51.68% and IVOMD % from 14.46 to 62.02%. Although large improvement of the BTF degradability was achieved, the biologically treated feathers (BTF) still less degradable than soybean meal. Soybean meal recorded the highest ($p<0.05$) percentage of IVDMD (68.64%) and IVOMD (73.39%). Accordingly, the use of crude feathers as substitutes for soybean meal in ruminant's rations has been excluded in the subsequent *in vitro* study. There was non-significant difference in chemical composition of the supplemented rations with BTF (R_{10}, R_{20} and R_{30}) than control ration (Table 3).

In the second *in vitro* study, effects of partial substitution of control ration's soybean meal by 10, 20 and 30% (R_{10}, R_{20} and R_{30}) of BTF on rumen fermentation characteristics were illstrated in Table 4. Ruminal dry matter and neutral detergent fiber degradability (%), pH value, ammonia-nitrogen and total volatile fatty acids concentrations did not differ among all treatments. But, organic matter and acid detergent fiber degradability (%), SCFA concentration and total gas production volume (mL/24 h) showed slight decrease when BTF substitution level increase. The OM and ADF degradability (%), SCFA concentration and TGP volume (mL/24 h) tended to be greater ($p<0.05$) in the control ration than those of R_{30}. while, there were no significant differences between control ration and R_{10} and R_{20} in all of rumen fermentation characteristics.

DISCUSSION

Diverse fungal, bacterial and actinomyces strains are able to secrete keratinolytic enzymes. The availability of such strains makes the selection of fast growing strain with high productivity and safety is an important step[28].

The time course productions of keratinase by fungal strains under shaking condition were reported by other researchers and indicated that the maximum yield were obtained within 3-7 days[29]. In this concern, a trial conducted by Noronha *et al.*[30] on *Aspergillus fumigates* indicated that the maximum keratinase production yield was obtained after only 3 days of incubation. Whereas, four days of incubation were reported by Gradisar *et al.*[31] and Da Gioppo *et al.*[32] as the optimum for keratinase production. Meanwhile, incubation of *Penicillium* sp. for five days was found to be the optimum for keratinase production[33]. On the other hand, Saber *et al.*[29] observed that *Aspergillus nidulans* K7, *Alternaria tenuissima* K2 and *Fusarium culmorum* produce the maximum keratinase yield after 5, 6 and 7 days of cultivation respectively.

With respect to the keratinase production by actinomyces strains, it had been observed by Gousterova *et al.*[34] that the

optimum production of keratinase by *Thermoactinomyces* sp. was observed after five days of incubation. However, the keratinase production by thermophilic *Streptomyces thermoviolaceus* SD8 was observed after only 24 h of incubation[35].

Other researchers also recorded different groups of bacteria able to produce keratinase, but Gram positive strains were the most effective ones. Bacterial species belong to *Bacillus* (*B. licheniformis*, *B. pumilus* and *B. subtilis*) were frequently used in industrial application due to its ability to secrete huge amount of keratinase[3,36,37]. De Boer *et al.*[38] reported that since 1972, *B. licheniformis* was used in large scale production of food processing enzymes. Consequently, it had been listed as safe and non-pathogenic source of proteases in the 3rd edition of Food Chemicals Codex (1981).

The decrease in *B. licheniformis* ALW1 keratinase production after reaching its optimum could be explained by the foundation that the production of the bacterial keratinase was mainly matched with the growth curve[3].

The quality of the biologically treated feathers (BTF) was checked by amino acids analysis. It was obvious that BTF quality had been increased as some of essential-sulphur amino acids like methionine and histidine had been improved on it. It is well known that essential-sulphur amino acids are playing an important role for development of animal's productive performance. The results indicated that, the microbial biomass with the degraded keratin may serve as a good protein source for ruminants. Moreover, the BTF may supply ruminant's diets with ruminal escape methionine. Richardson and Hatfield[39] reported that methionine is the first limiting amino acids in microbial protein for ruminant's growth. So such addition of BTF to ruminant's diets may cover animals need from sulphur amino acids and consequently improve their productive performance. In the current study, amino acids content of BTF using *B. licheniformis* ALW1 were comparable to their contents in the treated feather with keratinolytic bacteria *Vibrio* kr6[12] and *Bacillus licheniformis* ER-15[13] except for few quantitative differences.

Concerning with the chemical composition of feed ingredients, the reduction of CP, NDF and ADF percentage in BTF compared to their percentages in crude feather is reasonable due to the hydrolytic action of the *Bacillus licheniformis* ALW1 and absence of compact keratin, which were removed by filtration. The differences between crude feather and BTF in ash, EE and NFC contents may be explained by composition of *Bacillus licheniformis* ALW1 culture medium. This medium had mineral salts, which may increased BTF ash content. Also, it contained rich source of carbohydrates and oils (corn steep liquor) which may caused increase of BTF contents of EE and NFC. Current findings for BTF's DM, CP and EE percentages were lower than percentages in the fermented feather meal by *Micrococcus roseus*[1]. In contrast, BTF's ash content was higher than ash content of *Micrococcus roseus* treated feathers. The chemical composition variations of different fermented feather products can be attributed to differ in raw materials of fermentation medium and different techniques used for feather processing.

Utilization of feather meal as replacer for soybean meal in rations of ruminants has been studied. The current results were in line with those obtained by Suttiniyom *et al.*[40], who found increase in DM, CP and ash contents with slight decrease in crude fiber content of silage supplemented with 5, 10 and 15% of digested feather by *Bacillus subtilis* G8. Also Cozzi *et al.*[41] matched current results when replace 44% of soybean meal protein by feather and blood meal in rations of sheep. They stated that feather and blood meal inclusion led to slight increase in DM, CP and ash contents of the treated rations with quiet reduction in NDF, ADF and EE contents. The chemical composition changes of the supplemented rations with BTF (R_{10}, R_{20} and R_{30}) than control ration can be attributed to difference chemical composition of BTF and soybean meal (Table 3). The greater IVDMD % and IVOMD % for BTF than crude feather suggesting more efficient utilization of the BTF protein content by rumen microorganisms. This means that the solubility of BTF protein was improved due to action of *Bacillus licheniformis* ALW1. The superior of soybean meal over BTF in DM and OM degradability %, probably due to a greater availability of NFC on it than its availability in BTF (Table 2), Also, it had been reported that protein digestibility of feather meal was less than that of soybean meal[42]. Gas production volume and SCFA concentration differences between control and R_{30} might be due to the difference in carbohydrate content of each of them. Coelho *et al.*[43] confirmed positive correlation between ration's carbohydrates content and the volume of gas production during process of microbial fermentation in the rumen. Also Palizdar *et al.*[44] reported that animal origin feeds had lower gas production at 24 h incubation time than plant origin source. Lower NDF, ADF and NFC (carbohydrate fraction) and higher ash contents in BTF may be lead to a lower gas and SCFA production and OM and ADF degradability of the rations containing BTF (R_{10}, R_{20} and R_{30}).

CONCLUSION

Bacillus licheniformis ALW1 was the most potent tested microorganism for production of feather protein hydrolysate.

Utilization of *Bacillus licheniformis* ALW1 treated feather as substitute for costly soybean meal in ruminant's rations up to 20% had no negative effect on all of rumen fermentation characteristics. *In vivo* studies should be conducted to evaluate BTF utilization as economic protein source for ruminants feeding in the future.

SIGNIFICANCE STATEMENT

This study discover the possibility of using BTF as alternative feedstuff for high cost soybean meal in ruminant's rations through studying effect of BTF inclusion on rumen fermentation characteristics (*in vitro*). This study will help the ruminant's breeders to use of cheap and available BTF as a replacer for soybean meal at the optimum level which will reduce their animal's feeding cost and maximizing their profits.

REFERENCES

1. Bertsch, A. and N. Coello, 2005. A biotechnological process for treatment and recycling poultry feathers as a feed ingredient. Bioresour. Technol., 96: 1703-1708.
2. Chaturvedi, V., K. Bhange, R. Bhatt and P. Verma, 2014. Production of kertinases using chicken feathers as substrate by a novel multifunctional strain of *Pseudomonas stutzeri* and its dehairing application. Biocatal. Agric. Biotechnol., 3: 167-174.
3. Ire, F.S. and A.C. Onyenama, 2017. Effects of some cultural conditions on keratinase production by *Bacillus licheniformis* strain NBRC 14206. J. Adv. Boil. Biotechnol., 13: 1-13.
4. Williams, C.M., C.G. Lee, J.D. Garlich and J.C.H. Shih, 1991. Evaluation of a bacterial feather fermentation product, feather-lysate, as a feed protein. Poult. Sci., 70: 85-94.
5. El-Ghonemy, D.H. and T.H. Ali, 2017. Optimization of physico-chemical parameters for hyper keratinase production from a newly isolated sp. DHE7 using chicken feather as substrate-management of biowaste. J. Applied Pharm. Sci., 7: 171-178.
6. Huston, J.E. and M. Shelton, 1971. An evaluation of various protein concentrates for growing finishing lambs. J. Anim. Sci., 32: 334-338.
7. Wray, M.I., W.M. Beeson, T.W. Perry, M.T. Mohler and E. Baugh, 1979. Effect of soybean, feather and hair meals and fat on the performance of growing-finishing beef cattle. J. Anim. Sci., 48: 748-757.
8. Church, D.C., D.A. Daugherty and W.H. Kennick, 1982. Nutritional evaluation of feather and hair meals as protein sources for ruminants. J. Anim. Sci., 54: 337-344.
9. Lu, C.D., M.J. Potchoiba, T. Sahlu and J.R. Kawas, 1990. Performance of dairy goats fed soybean meal or meat and bone meal with or without urea during early lactation. J. Dairy Sci., 73: 726-734.
10. Wang, X. and C.M. Parsons, 1997. Effect of processing systems on protein quality of feather meals and hog hair meals. Poult. Sci., 76: 491-496.
11. Gang, G., C. Jie, W. Jun-gao, H. Qiu-xia and L. Ke-chun, 2013. A two-step biotechnological process for improving nutrition value of feather meal by *Bacillus licheniformis* S6. J. Northeast Agric. Univ., 20: 71-77.
12. Grazziotin, A., F.A. Pimentel, E.V. de Jong and A. Brandelli, 2006. Nutritional improvement of feather protein by treatment with microbial keratinase. Anim. Feed Sci. Technol., 126: 135-144.
13. Tiwary, E. and R. Gupta, 2012. Rapid conversion of chicken feather to feather meal using dimeric keratinase from *Bacillus licheniformis* ER-15. J. Bioproc. Biotech, Vol. 2. 10.4172/2155-9821.1000123.
14. Abdel-Fattah, A.M., E.S.H. Nashy, E.T.A. Sabiel, M.M. Hussien and A.S. Attia, 2015. Novel keratinolytic activity of *Cyberlindnera fabianii* Nrc3 Aza as a Plant Growth Promoting Agent (PGPA). Int. J. Applied Sci. Biotechnol., 3: 609-618.
15. Wang, J.J. and J.C.H. Shih, 1999. Fermentation production of keratinase from *Bacillus licheniformis* PWD-1 and a recombinant *B. subtilis* FDB-29. J. Indus. Microbiol. Biotechnol., 22: 608-616.
16. Cai, C.G., B.G. Lou and X.D. Zheng, 2008. Keratinase production and keratin degradation by a mutant strain of *Bacillus subtilis*. J. Zhejiang Univ. Sci. B, 9: 60-67.
17. AOAC., 2012. Official methods of analysis of the AOAC international No. 994.12. Edn., Chapter 4, 18-19, Official Journal of the European Communities 19.9.98, Gaithersburg, Maryland, USA.
18. AOAC., 1995. Official Methods of Analysis of AOAC International. 16th Edn., Vol. 1, Association of Official Agricultural Chemists International, Arlington, Virginia, USA.
19. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.
20. Tilley, J.M.A. and R.A. Terry, 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Sci., 18: 104-111.
21. El-Sherbiny, M., A. Cieslak, E. Pers-Kamczyc, J. Szczechowiak, D. Kowalczyk and M. Szumacher-Strabel, 2016. A nanoemulsified form of oil blends positively affects the fatty acid proportion in ruminal batch cultures. J. Dairy Sci., 989: 399-407.

22. Navarro-Villa, A., M. O'Brien, S. Lopez, T.M. Boland and P. O'Kiely, 2011. Modifications of a gas production technique for assessing *in vitro* rumen methane production from feedstuffs. Anim. Feed Sci. Technol., 166-167: 163-174.
23. Szczechowiak, J., M. Szumacher-Strabel, M. El-Sherbiny, E. Pers-Kamczyc, P. Pawlak and A. Cieslak, 2016. Rumen fermentation, methane concentration and fatty acid proportion in the rumen and milk of dairy cows fed condensed tannin and/or fish-soybean oils blend. Anim. Feed Sci. Technol., 216: 93-107.
24. Warner, A.C., 1964. Production of volatile fatty acids in the rumen: Methods of measurement. Nutr. Abstr. Rev., 34: 339-352.
25. Makkar, H.P.S., 2005. *In vitro* gas methods for evaluation of feeds containing phytochemicals. Anim. Feed Sci. Technol., 123-124: 291-302.
26. SPSS., 2008. Statistical Package for Social Sciences, Statistics for Windows. Version, 17.0, SPSS Inc., Chicago, USA.
27. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
28. Kanchana, R., 2012. Farm waste recycling through microbial keratinases. J. Applied Sci. Environ. Sanit., 7: 103-108.
29. Saber, W.I.A., M.M. El-Metwally and M.S. El-Hersh, 2010. Keratinase production and biodegradation of some keratinous wastes by *Alternaria tenuissima* and *Aspergillus nidulans*. Res. J. Microbiol., 5: 21-35.
30. Noronha, E.F., B.D. de Lima, C.M. de Sa and C.R. Felix, 2002. Heterologous production of *Aspergillus fumigatus* keratinase in *Pichia pastoris*. World J. Microbiol. Biotechnol., 18: 563-568.
31. Gradisar, H., S. Kern and J. Friedrich, 2000. Keratinase of *Doratomyces microsporus*. Applied Microbiol. Biotechnol., 53: 196-200.
32. Da Gioppo, N.M.R., F.G. Moreira-Gasparin, A.M. Costa, A.M. Alexandrino, C.G.M. de Souza and R.M. Peralta, 2009. Influence of the carbon and nitrogen sources on keratinase production by *Myrothecium verrucaria* in submerged and solid state cultures. J. Ind. Microbiol. Biotechnol., 36: 705-711.
33. El-Gendy, M.M.A., 2010. Keratinase production by endophytic *Penicillium* spp. Morsy1 under solid-State fermentation using rice straw. Applied Biochem. Biotechnol., 162: 780-794.
34. Gousterova, A., D. Braikova, I. Goshev, P. Christov and K. Tishinov *et al*., 2005. Degradation of keratin and collagen containing wastes by newly isolated thermoactinomycetes or by alkaline hydrolysis. Lett. Applied Microbiol., 40: 335-340.
35. Chitte, R.R., V.K. Nalawade and S. Dey, 1999. Keratinolytic activity from the broth of a feather-degrading thermophilic *Streptomyces thermoviolaceus* strain SD8. Lett. Applied Microbiol., 28: 131-136.
36. Sahni, N., P.P. Sahota and U.G. Phutela, 2015. Bacterial keratinases and their prospective applications: A review. Int. J. Curr. Microbiol. Applied Sci., 4: 768-783.
37. Chaisemsaeng, P., A. Sabu, S. Ansanan and S. Sirisan, 2017. Feather degradation and keratinase production by *Bacillus* sp. and *Lactobacillus* sp. Int. J. Biotechnol. Res., 7: 29-36.
38. De Boer, A.S., F. Priest and B. Diderichsen, 1994. On the industrial use of *Bacillus licheniformis*: A review. Applied Microbiol. Biotechnol., 40: 595-598.
39. Richardson, C.R. and E.E. Hatfield, 1978. The limiting amino acids in growing cattle. J. Anim. Sci., 46: 740-745.
40. Suttiniyom, C., S. Yammuen-Art, A. Kanpiengjai, K. Unban and C. Khanongnuch, 2015. Digestibility and protein content improvement of corncob silage using chicken feather partially digested by *Bacillus subtilis* G8. Int. J. Agric. Biol., 17: 1207-1212.
41. Cozzi, G., I. Andrighetto, P. Berzaghi and D. Andreoli, 1995. Feather and blood meal as partial replacer of soybean meal in protein supplements for sheep. Small Rumin. Res., 15: 239-245.
42. Thomas, V.M., C.K. Clark and C.M. Schuldt, 1994. Effects of substituting feather meal for soybean meal on ruminal fiber fermentation and lamb and wool growth. J. Anim. Sci., 72: 509-514.
43. Coelho, M., F.G. Hembry, F.E. Barton and A.M. Saxton, 1988. A comparison of microbial, enzymatic, chemical and near-infrared reflectance spectroscopy methods in forage evaluation. Anim. Feed Sci. Technol., 20: 219-231.
44. Palizdar, M.H., H. Sadeghipanah, H. Amanlou, K. Nazer and A. Mirhad, 2011. *In vitro* organic matter digestibility and gas production of fish-meal coated with fat. Afr. J. Biotechnol., 10: 248-255.