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Production of an environmentally friendly enzymatic feed additive for agriculture animals by spray drying abattoir's rumen fluid in the presence of different hydrocolloids

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ABSTRACT

The potential to use abattoir's rumen fluid as a source to produce a carbohydrate degrading enzymatic feed additive by using spray-drying technique was studied. Rumen contents were taken from the slaughterhouse and powdered by spray drying with different hydrocolloids including sodium alginate (RA), guar gum (RG), chitosan (RC) and maltodextrin (RM) in two ratios (0.5 and 1% (w/v)). Fresh (RF) and spray dried rumen fluid without hydrocolloid materials (RN) were considered as controls. Residual activities compared to those measured in the fresh rumen fluid ranged from 68.6 (RC0.5) to 92.5% (RM1) for carboxymethyl cellulase, from 53.4 (RC1) to 73.2% (RM1) for avicelase, from 59.8 (RA0.5) to 84.6% (FM1) for amylase, and from 63.7 (RG0.5) to 95.8% (RM1) for filter paperase. Spray drying in the absence of a hydrocolloid resulted in 81.3% residual activity of carboxymethyl cellulase, 63.3% of avicelase, 68.6% of amylase, and 73.0% of filter paperase. The addition of 1% (w/v) maltodextrin was shown to retain the highest enzyme activities after spray drying. In addition, a dry matter degrading test was carried out to show the ability of the enzyme preparations at two concentrations (1 or 2% solution in phosphate buffer) to digest a typical dairy cow diet. At 1%, RF resulted in highest dry matter digestibility ($P < 0.05$) and at 2% dry matter digestibility of RC0.5, RC1 and RF were similar ($P < 0.05$). With increasing amounts of the enzyme preparations, an increase in dry matter digestibility occurred ($P < 0.05$). This study suggests that use spray drying technique with additives especially maltodextrin could be considered as an efficient method for drying abattoir's rumen fluid to produce an environmental friendly enzyme additive for animal feeding.

1. Introduction

Rumen contents, one of the byproducts of slaughterhouses, are commonly considered as waste materials creating environmental pollution due to their ammonia and phosphorous contents (Tritt and Schuchardt, 1992). Their high moisture content is a major obstacle in the slaughterhouses requiring an appropriate processing. The amount of rumen content varies with ruminant type and body weight and on average it was reported to be 10 kg per animal for small ruminants and 40 kg for large ruminants (Afazeli et al., 2014; Abdeshahian et al., 2016). Each cubic meter of rumen content consists of 0.5–0.6 m^3 liquid phase (Tritt and Schuchardt, 1992). This liquid phase, called rumen fluid, derived daily from millions of ruminant animals slaughtered around the world is not utilized and simply released into the environment. The existing system of disposing abattoir's rumen wastes causes not only problems of odor, flies and hygiene, but also pollution of surface and ground waters with pathogens and undesirable chemical compounds (Yitbarek et al., 2016).

Ruminant animals live in symbiosis with rumen microorganisms in order to utilize fibrous feeds as a nutrient source (Infascelli

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et al., 2005). The diversity of rumen microbes includes bacterial, protozoal and fungal species and a diverse microbial community in the rumen led to a high variety of secreted enzymes. Those enzymes are responsible for an efficient hydrolysis of the complex substrates in the rumen. Rumen is therefore a rich source of enzymes, especially polysaccharide degrading enzymes, among others amylases, cellulases and xylanases (Yue et al., 2013). Yue et al. (2013) already proposed that rumen might be a novel source of useful enzyme for industrial applications. Furthermore, rumen fluid could be a source of metabolites of microbial activities among others proteins, amino acids, vitamins and volatile fatty acids.

Spray drying is generally used by industry for decreasing the water content and water activity of a product to ensure its microbiological stability, to avoid the risk of chemical and or biological degradation, to reduce storage and transport costs, and finally to obtain a product with specific properties (Samborska et al., 2005; Gharsallaoui et al., 2007). Spray drying is the most common method for drying of liquids in the dairy and pharmaceutical industries. It is used for dehydration of various substances such as milk, whey, antibiotics, vitamins, and enzymes. The process uses a hot gas, generally air or more rarely an inert gas such as nitrogen, to obtain a powder. As a liquid, a solution, an emulsion or a suspension could be used (Samborska et al., 2005).

Due to their complex biochemical structures, enzymes are prone to rapid inactivation or degradation. The use of microcapsular delivery systems is one strategy to overcome the stability problem of enzymes and therefore increasing the range of their applications (Shahidi and Han, 1993). The encapsulation matrix protects the enzymes from adverse environmental conditions and triggers their release at the target sites of application (de Vos et al., 2010). Biopolymers among others carbohydrates, gums and proteins of different sources are often used to encapsulate feed or food ingredients (Gharsallaoui et al., 2007). Spray drying is simple, fast and economic to perform and also applicable for temperature-sensitive compounds such as enzymes (Namaldi et al., 2006). Therefore, it is a widely applied technology for encapsulation.

Exogenous enzymes are commonly used as feed additives in non-ruminant and ruminant animals in order to improve feed digestibility, animal health and production efficiency (Meale et al., 2014; Menezes-Blackburn and Greiner, 2015). Rumen contents have been already proposed as fertilizer (Tritt and Schuchardt, 1992) or a source for biogas production (Afazeli et al., 2014). In addition, some efforts have been made to use dry rumen contents in animal feeding (Cherdthong et al., 2014). To the best of our knowledge, no report on the use of rumen fluid as a source to produce an enzymatic feed additive by spray drying is available. Therefore, the possibility to produce an enzymatic feed additive with abattoir's rumen fluid as a source material by spray drying in the presence of different hydrocolloids was evaluated and the resulting enzyme preparations were characterized.

2. Materials and methods

2.1. Sample preparation and spray drying

Rumen contents taken from a slaughterhouse (Ardabil industrial meat complex, Ardabil, Iran) were transported in pre-warmed containers to the laboratory. A laboratory blender under constant CO_2 purging was used to obtain a homogeneous mixture. In order to separate rumen fluid from rumen solid materials filtration through four-layers cheesecloth was used. The collected rumen fluid were spray dried by using different hydrocolloids including sodium alginate (Sigma-Aldrich, CAS Number, 9005-38-3, chemical Book), guar gum (Sigma-Aldrich, CAS Number, 9003-30-0), chitosan (Sigma-Aldrich, CAS Number, 9012-76-4, Tokyo chemical Industry Co. Ltd), and maltodextrin (Sigma-Aldrich, CAS Number, 9050-36-6, SCBT-Santa Cruz Biotechnology) in two ratios 0.5 and 1% (w/v). Fresh rumen fluid and spray dried rumen fluid without added hydrocolloids were considered as controls. The experimental groups were: 1) spray dried rumen fluid with 0.5% maltodextrin (RM0.5), 2) spray dried rumen fluid with 1% maltodextrin (RM1), 3) spray dried rumen fluid with 0.5% chitosan (RC0.5), 4) spray dried rumen fluid with 1% chitosan (RC1), 5) spray dried rumen fluid with 0.5% guar gum (RG0.5), 6) spray dried rumen fluid with 1% guar gum (RG1), 7) spray dried rumen fluid with 0.5% alginate (RA0.5), 8) spray dried rumen fluid with 1% alginate (RA1), 9) spray dried rumen fluid with no hydrocolloid (RN), and 10) fresh rumen fluid (RF). A laboratory scale spray dryer (Armfield Mini Spray Dryer, England) with two-fluid nozzles (inner diameter: 0.5 mm) was used for spray drying. The system was operated in a co-current manner with an inlet and outlet air temperature of 120 °C and 50 °C, respectively. Feed rate change in 240–640 mL/h was necessary to achieve a constant outlet temperature. A spray flow rate of 500 L/h was used and the aspiration rate was 70%. The powdered samples were stored in two layers polyester bags and kept in the refrigerator (5 °C) until analysis.

2.2. Enzyme activities measurement

For determination of enzyme activities, 1 g of each powdered sample was dissolved in 100 mL 0.1 M phosphate buffer, pH 6.8 (buffer A). To determine carboxymethyl cellulase activity (CMCase), 0.5 mL of the enzyme-containing sample solution was thoroughly mixed with 1 mL buffer A and 0.5 mL carboxymethyl cellulose solution (1 g carboxymethyl cellulose dissolved in 100 mL distilled water). Microcrystalline cellulase activity (Avicelase) was determined by mixing 1 mL of the enzyme-containing sample solution with 1 mL microcrystalline cellulose (Avicel, Sigma-Aldrich, CAS Number, 9004-34-6) solution (1 g avicel dissolved in 100 mL buffer A) and 1 mL buffer A. The mixtures were kept for 60 min at 39 °C. In order to determine amylase activity, 0.25 mL of the enzyme-containing sample solution was thoroughly mixed with 0.5 mL buffer A and 0.25 mL starch (Sigma-Aldrich, CAS Number, 9005-25-8, SCBT-Santa Cruz Biotechnology) solution (1 g starch dissolved in 100 mL distilled water). Thereafter, incubation at 39 °C for 30 min was performed. Filter papers activity (Ftpase) was determined according to Agarwal et al. (2000). One mL of the enzyme-containing sample solutions were mixed with 1 mL distilled water and 1 mL buffer A. Thereafter, 50 mg Whatman filter paper strip (No. 1) was added and the mixtures were kept at 39 °C for 1 h. To stop all the enzymatic reactions, 3 mL dinitrosalycylic acid solution (10 g dissolved in 500 mL 2% sodium hydroxide solution) were added and the mixtures were incubated at 100 °C for 10 min in a water bath. After adding, 1 mL Rochelle salt solution (40 g Rochelle salt dissolved in 100 mL distilled water), the mixtures were cooled down under running tap water. Finally, absorbance at 575 nm was recorded (Spectrophotometer, UNICO 2100). In order to quantify the glucose released, a standard curve with glucose was used. 1 U of enzyme activity was defined as μmole of reducing sugars produced per minute per ml under assay conditions. All enzyme activity determinations were performed in triplicate. Residual enzyme activities of the spray-dried samples were presented as percentage of the enzyme activity of the fresh rumen fluid.

2.3. Dry matter digestibility

Dry matter digestibility was determined according to Holden (1999). Each spray dried rumen fluid powder including the dried samples with and without hydrocolloids were reconstituted by

dissolving in buffer A using two concentrations (1 or 2% w/v). Then, a typical dairy cow diet (25% alfalfa hay, 25% corn silage, 10% corn grain, 15.5% barley grain, 18.3% wheat bran, 5% calcium carbonate, 0.5% vitamin premix, 0.5% mineral premix, and 0.2% sodium chloride) was grounded with a laboratory mill (1 mm screen) and incubated with each of the reconstituted spray dried rumen fluid solutions. 15 mL of reconstituted rumen fluid solution mixed with 15 mL of McDougall's buffer were added to 1 g of dairy cow diet in a 100 mL Erlenmeyer flask. The suspension was kept at 39 °C for 24 h under agitation. Thereafter, the residues were filtered by one layer polyester cheesecloth (52 ± 5 μm pore size, Gol Pooneh Safahan, Isfahan, Iran) and dried at 65 °C for 48 h and dry matter losses were considered as dry matter digestibility. The potential of the enzyme preparations to digest the diet was calculated as the dry matter losses of the diet in the presence of the enzyme preparation corrected for the dry matter losses of the diet when the enzyme preparation was substituted by distilled water.

2.4. Statistical analysis

The GLM procedure of SAS (SAS, 2003) was used for data analysis as randomized complete design according to the following model:

$$Y_{ij} = \mu + T_i + e_{ij} \quad (1)$$

Where Y represents the dependent variable, μ the overall mean, T_i the effect of the processing method and e the random error. Comparison between experimental groups was done by Tukey test and significant differences were defined at $P < 0.05$.

3. Results

3.1. Enzymatic activities of the spray dried enzyme preparations

The main polysaccharide degrading enzyme activities in the fresh rumen fluid were determined to be 79.9 U/mL for CMCase activity, 39.9 U/mL for avicelase activity, 857.6 U/mL for amylase activity and 91.9 U/mL for Ftpase activity. The residual enzyme activities after spray drying in the presence and absence of hydrocolloids are shown in Table 1. Spray drying of the rumen fluid in the absence of a hydrocolloid resulted in a loss of enzyme activity for all enzymes studied. The stabilizing effect of a hydrocolloid during spray drying could not always be observed. In addition, increasing the hydrocolloid concentration from 0.5 to 1% did not always result in higher residual enzyme activities. The highest residual activities for all polysaccharide-degrading enzymes studied were obtained in the presence of 1% (w/v) maltodextrin during spray drying.

Table 1
Residual enzyme activities (%) of the spray dried enzyme preparations.

Treatment	CMCase	Avicelase	Amylase	Ftpase
RM0.5	80.54 ± 4.20^{cd}	67.64 ± 4.44^{ab}	75.74 ± 5.35^{b}	87.84 ± 1.25^{c}
RM1	92.45 ± 5.45^{a}	73.20 ± 1.39^{a}	84.57 ± 4.76^{a}	95.77 ± 1.83^{a}
RC0.5	68.60 ± 1.89^{f}	62.55 ± 6.57^{bcd}	65.96 ± 3.41^{c}	73.82 ± 1.11^{f}
RC1	84.93 ± 1.08^{bc}	53.42 ± 1.06^{e}	63.66 ± 2.32^{cd}	91.83 ± 4.42^{b}
RG0.5	71.43 ± 3.02^{ef}	66.86 ± 4.85^{ab}	66.25 ± 1.81^{c}	63.70 ± 0.06^{h}
RG1	69.85 ± 5.07^{f}	58.05 ± 5.46^{cde}	69.77 ± 1.98^{c}	68.75 ± 2.21^{g}
RA0.5	90.72 ± 2.61^{ab}	54.45 ± 5.01^{de}	59.77 ± 1.16^{d}	84.12 ± 1.45^{d}
RA1	77.08 ± 2.12^{de}	54.87 ± 3.20^{de}	64.29 ± 4.35^{cd}	79.18 ± 2.13^{e}
RN	81.30 ± 5.86^{cd}	63.26 ± 4.47^{bc}	68.58 ± 1.11^{c}	72.99 ± 1.14^{f}
P value	<0.0001	<0.0001	<0.0001	<0.0001

Spray dried rumen fluid with 0.5% (RM0.5) and 1% (RM1) maltodextrin, with 0.5% (RC0.5) and 1% (RC1) chitosan, with 0.5% (RG0.5) and 1% (RG1) guar gum, with 0.5% (RA0.5) and 1% (RA1) alginate, and with no hydrocolloids added (RN). The enzyme activities are given in % of those obtained in the fresh rumen fluid as mean ± slandered error.
Different superscripts indicate significant differences in a column.
Significance level was considered at $P < 0.05$.

3.2. Dry matter-degrading test

The potential of experimental samples including fresh rumen fluid and spray dried rumen fluid with or without hydrocolloids at two dissolving conditions (1 or 2 percent solution in phosphate buffer) for digesting a typical dairy cow diet are presented in Figs. 1 and 2. Incubation of the dairy cow diet with 1% RF resulted in a higher dry matter digestibility compared to the spray dried preparations ($P < 0.05$) (Fig. 1). At 1% dissolving condition, no significant differences among RM0.5, RM1, RG0.5, RG1, RA1 and RN were observed. The lowest dry matter digestibility of the diet was obtained for RC0.5. At 2%, RC0.5 and RC1 were comparable to RF in digesting dry matter of the diet (Fig. 2). All other enzyme preparations showed lower dry matter digestibility ($P < 0.05$).

4. Discussion

Environment pollution is a matter of growing concern daily (Abdeshahian et al., 2016). However, rumen fluid is a slaughterhouse waste exhibiting problems for its disposal and it also causes environmental pollution (Tritt and Schuchardt, 1992). In this study, the potential to produce an enzymatic feed additive using rumen fluid as an enzyme source by spray drying technique in the presence of selected hydrocolloids was evaluated. Since enzymes are prone to denaturation by heat, long term exposure to high temperatures need to be avoided while drying enzymes (Namaldi et al., 2006). Starting with a liquid medium, spray drying uses a hot gas to produce in a rapid process a dry powder and is the method preferred to dry temperature sensitive compounds (Samborska et al., 2005). Because of the cooling effect due to the rapid water evaporation, the droplet temperature is maintained low (Stahl et al., 2002).

Spray drying rumen fluid in the absence of hydrocolloids resulted in a loss of polysaccharide degrading enzyme activities (Table 1). Dehydration by spray drying may cause changes in the three-dimensional protein structure similar to a temperature-induced denaturation and may result in a complete loss of enzymatic activity. The change in the three dimensional structure of a protein is usually less likely in the presence of compounds such as polysaccharides, proteins or salts (Alloue et al., 2007). Furthermore, shear forces occurring in the spray nozzle or adsorption of enzymes at the surface of droplets might result in denaturation and loss of

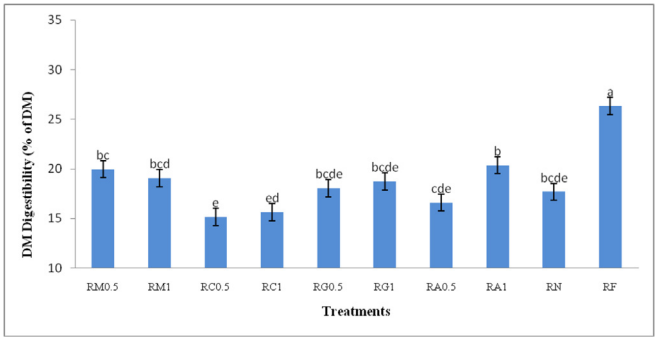

Fig. 1. Dry matter digestibility of a typical dairy cow diet by different ruminal fluid preparations at 1% dissolving rate. Different superscripts indicate significant differences ($P < 0.05$).

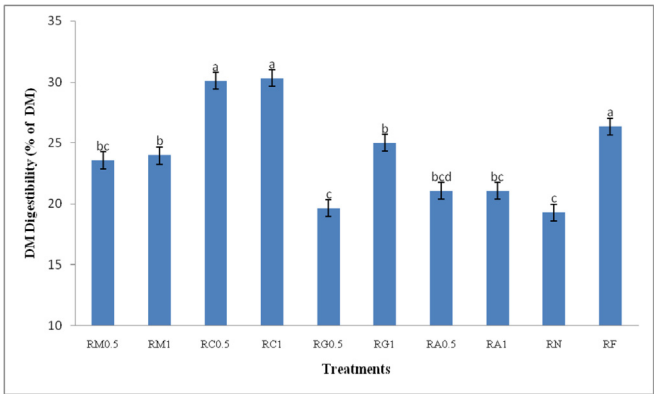

Fig. 2. Dry matter digestibility of a typical dairy cow diet by different ruminal fluid preparations at 2% dissolving rate. Different superscripts indicate significant differences ($P < 0.05$).

enzyme activity (Gharsallaoui et al., 2007). A protective effect of hydrocolloids in respect to thermal denaturation was already reported (Schule et al., 2008). Polysaccharides are an excellent choice for stabilizers and carrier material because of their stability, their wide abundance in nature and their low price (Fathi et al., 2014). Among the hydrocolloids studied, enzyme activity remained highest in the presence of maltodextrin during spray drying (Table 1). In accordance with the results obtained in this study, Alloue et al. (2007) also identified maltodextrin as the best stabilizer. They observed that addition of compounds like skim milk powder, gum arabic, maltodextrin, and calcium chloride were capable of stabilizing lipase during spray drying and up to a 1.46-fold higher lipase activity was reported in the presence of those compounds compared to a control without any stabilizers. There are different mechanisms discussed how hydrocolloids stabilize the enzymes during the spray drying process. A direct interaction of the hydrocolloids with the enzymes was proposed, stabilizing the enzyme structure by formation of hydrogen bonds (Lauruengtana et al., 2009). In addition, hydrocolloids may act as a water trap close to the surface of the enzymes or they act by an entrapment of a particular protein conformation in a highly viscous amorphous glassy matrix (Lauruengtana et al., 2009). Both mechanisms mentioned in the previous sentence have been proposed for maltodextrin by DePaz et al. (2002).

Rumen fluid contains different microorganisms producing a complex enzyme mixture capable of degrading complex macromolecules including proteins, carbohydrates and lipids. Simple *in vitro* enzyme activity assays are used to quantify the enzymatic activity of a protein using model synthetic substrates or at least not the *in vivo* substrate of the enzyme. However, these assays do not represent the situation in the digestive tract of an animal and are therefore only of little relevance in respect of the usefulness of a certain enzyme as a feed additive. A better approach to evaluate the biological efficacy of an enzyme product containing different enzymatic activities would be its incubation with complex (e.g. a typical animal diet) rather than purified substrates. In this study, the degrading potential of the ruminal fluid preparations compared to the fresh rumen fluid as an enzyme source was investigated by an *in vitro* dry matter degrading test (Figs. 1 and 2). Based on the results obtained, spray dried ruminal preparations when reconstituted in phosphate buffer showed the ability to digest a typical dairy cow diet. Reconstituted solutions had lower digesting ability at 1% (w/v) dissolving condition compared to fresh rumen fluid. This observation is in accordance with enzyme assays data in which lower residual enzyme activities have been observed for spray-dried samples (Table 1). When adding the enzyme preparations at 2% (w/v) dissolving condition to the cow diet was tested, a comparable dry matter digestibility to the fresh rumen fluid was obtained. This data suggest that the dried ruminal preparations remain active after spray drying and rumen fluid keeps its enzymatic ability for digesting complex feeds after the drying process. At 1% dissolving condition, higher dry matter digestibility was observed with RM0.5, RM1 and RA1 reconstituted solutions. The observed results about higher degrading ability of maltodextrin added spray-dried rumen fluid approve the results of enzyme assay experiment that maltodextrin added preparations had better enzyme activities.

The use of exogenous enzymes as feed additives is continuously increasing because their application improves feed efficiency in a cost effective manner (Meale et al., 2014). The commercially available enzyme products used as feed additives are of fungal or bacterial origin and commonly produced by microbial fermentation techniques. Yue et al. (2013) suggested the rumen fluid as a novel and unutilized enzyme source. Rumen fluid is also a source of nutrients such as microbial proteins, amino acids, vitamins and volatile fatty acids for livestock especially ruminants. Furthermore, the use of the waste product rumen fluid reduces its negative environmental effects related to its disposal.

5. Conclusion

Due to the existence of different microbial species, rumen fluid contains a wide spectrum of macromolecule-degrading enzymes. Spray drying fresh rumen fluid in the presence of maltodextrin was shown to make the production of a powder with high carbohydrate-degrading activities feasible. Because this enzyme preparation has, the ability to digest dry matter of a typical cow diet, it could be successfully used as a feed additive in order to improve feed efficiency. In conclusion, spray drying of abattoir's rumen fluid is considered as a suitable method to produce enzymes as an additive for animal feeding, and at the same time reduces the environment contamination. More work however is needed to develop an industrial scale process for managing rumen fluid wastes.

References

Abdeshahian, P., Lim, J.S., Ho, W.S., Hashim, H., Lee, C.T., 2016. Potential of biogas production from farm animal waste in Malaysia. Renew. Sustain. Energy Rev. 60, 714–723.

Afazeli, H., Jafari, A., Rafiee, S., Nosrati, M., 2014. An investigation of biogas production potential from livestock and slaughterhouse wastes. Renew. Sustain. Energy Rev. 34, 380–386.

Agarwal, N., Agarwal, I., Kamra, D.N., Chaudhary, L.C., 2000. Diurnal variations in the activities of hydrolytic enzymes in different fractions of rumen contents of Murrah buffalo. J. Appl. Anim. Res. 18 (1), 73–80.

Alloue, W.A.M., Destain, J., Amighi, K., Thonart, P., 2007. Storage of *Yarrowiali polytica* lipase after spray-drying in the presence of additives. Process Biochem. 42, 1357–1361.

Cherdthong, A., Wanapat, M., Saenkamsorn, A., Waraphila, N., Khota, W., Rakwongrit, D., Anantasook, N., Gunum, P., 2014. Effects of replacing soybean meal with dried rumen digesta on feed intake, digestibility of nutrients, rumen fermentation and nitrogen use efficiency in Thai cattle fed on rice straw. Livest. Sci. 169, 71–77.

DePaz, R.A., Dale, D.A., Barnett, C.C., Carpenter, J.F., Gaertner, A.L., Randolph, T.W., 2002. Effects of drying methods and additives on the structure, function, and storage stability of subtilisin: role of protein conformation and molecular mobility. Enzym. Microb. Technol. 31, 765–774.

de Vos, P., Faas, M.M., Spasojevic, M., Sikkema, J., 2010. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int. Dairy J. 20, 292–302.

Fathi, M., Martin, A., McClements, D.J., 2014. Nanoencapsulation of food ingredients using carbohydrate based delivery systems. Trends Food Sci. Technol. 39, 18–39.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R., 2007. Applications of spray drying in microencapsulation of food ingredients: an overview. Food Res. Int. 40, 1107–1121.

Holden, L.A., 1999. Comparison of methods of *in vitro* dry matter digestibility for ten feeds. J. Dairy Sci. 82, 1791–1794.

Infascelli, F., Bovera, F., Piccolo, G., D'Urso, S., Zicarelli, F., Cutrignelli, M.I., 2005. Gas production and organic matter degradability of diets for buffaloes. Ital. J. Anim. Sci. 4, 316–318.
Lauruengtana, V., Paramita, V., Neoh, T.L., Furuta, T., Yoshii, H., 2009. Encapsulation of enzymes by spray drying. Japan. J. Food. Eng. 10, 79–85.
Meale, S.J., Beauchemin, K.A., Hristov, A.N., Chaves, A.V., McAllister, T.A., 2014. Board-invited review: opportunities and challenges in using exogenous enzymes to improve ruminant production. J. Anim. Sci. 92, 427–442.
Menezes-Blackburn, D., Greiner, R., 2015. Enzymes used in animal feed: leading technologies and forthcoming developments. In: Cirillo, G., Gianfranco-Spizzirri, G., Lemma, F. (Eds.), Functional Polymers in Food Science. Scrivener Publishing LLC, pp. 47–73.
Namaldi, A., Calik, P., Uludag, Y., 2006. Effects of Spray Drying Temperature and Additives on the stability of serine alkaline protease powders. Dry. Techol. 24, 1495–1500.
Samborska, K., Witrowa-Rajchert, D., Gonçalves, A., 2005. Spray-drying of α-amylase-The effect of process variables on the enzyme inactivation. Dry. Technol. 23, 941–953.
SAS Institute, 2003. SAS User's Guide: Statistics, Release 9.1. SAS Inst. Inc, Cary, NC.
Schule, S., Schulz-Fademrecht, T., Garidel, P., Bechtold-Peters, K., Frieb, W., 2008. Stabilization of IgG1 in spray-dried powder for inhalation. Eur. J. Pharm. Biopharm. 69, 793–807.
Shahidi, F., Han, X.Q., 1993. Encapsulation of food ingredients. Crit. Rev. Food Sci. Nutr. 33, 501–547.
Stahl, K., Claesson, M., Lilliehorn, P., Linden, H., Backstorm, K., 2002. The effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation. Int. J. Pharm. 233, 227–237.
Tritt, W.P., Schuchardt, F., 1992. Materials flow and possibilities of treating liquid and solid wastes from slaughterhouses in Germany. A review. Bioresour. Technol. 41, 235–245.
Yitbarek, M.B., Mersso, B.T., Wosen, A.M., 2016. Effect of dried blood-rumen content mixture (DBRCM) on feed intake, body weight gain, feed conversion ratio and mortality rate of SASSO C44 broiler chicks. J. Livest. Sci. 7, 139–149.
Yue, Z.B., Li, W.W., Yu, H.Q., 2013. Application of rumen microorganisms for anaerobic bioconversion of lignocellulosic biomass. Bioresour. Technol. 128, 738–744.