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The species identification of bovine, porcine, ovine and chicken components in animal meals, feeds and their ingredients, based on COX I analysis and ribosomal DNA sequences

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ABSTRACT

The objective of the study was to develop a universal method for the species identification of bovine, porcine, ovine and chicken components using PCR. The proposed primers generate short amplicons of 90, 85, 67 and 66 bp for cattle, pigs, sheep and chickens respectively within the gene encoding COX1 in the case of ovine and porcine tissues, 12SrRNA for cattle, and 16SrRNA for chickens. The proposed primers only amplify the DNA of the species for which they were designed, and do not cross-react with the DNA of other species of animals and plants, the tissues of which could be the ingredients of feed mixtures or products used for food production. The use of short amplification products for the indicators allows for the highly effective species identification of animal components, both in raw samples and in samples processed at high temperature and pressure. The method developed is effective for a broad range of animal products such as lard, animal meals, pet foods, plasma, whey, milk substitute, and others. The PCR products obtained are species specific for both components in amounts of 0.1% and for 100% animal samples. Limit of quantification (LOD) for the meal contained in plant feeds and in animal feeds from other species is 0.08% for poultry meal and 0.09% for bovine, porcine and ovine meal. The specificity and high sensitivity of the indicators, as well as the universality and usefulness of the method regardless of the degree of processing, type and form of the source material are its greatest advantages.

1. Introduction

Global research on the species identification of animal products in fresh and processed foods and feeds has provided much valuable information on the identification of components from both domestic (Ekins et al., 2012; Fumière, Dubois, Baeten, von Holst, & Berben, 2006; Myers et al., 2010; Prado et al., 2007) and wild animals (Fajardo et al., 2007; Fajardo et al., 2008a, 2008b; Natonek-Wiśniewska & Słota, 2010; Natonek-Wiśniewska, Słota, & Kalisz, 2010; Rojas et al., 2009; Rojas et al., 2011). This research is particularly relevant where reliable information on the qualitative composition of products is required to avoid the possibility of adulteration. Adulterated food can not only have a lower nutritional value, but can also pose a threat to health and life through the presence of prohibited substances or allergenic components. Similarly, breeders should know the components of the feeds they use because of a ban on feeding animal meals to food animals, which was imposed with the aim of preventing BSE (Council Decision 2000/766/EC). Determining the species origin of food components is now a routine analytical method for inspecting the actual composition of a food or feed. The most important methods are ELISA (Ansfield, Reaney, & Jackman, 2000; Asensio, González, García, & Martín, 2008; Berrini, Tepedino, Borromeo, & Secchi, 2006; Kotoura et al., 2012), which is based on protein analysis, as well as sequencing (Girish et al., 2004) and polymerase chain reaction, which are based on DNA identification. While the methods involving protein identification work well and are acceptable for the analysis of raw tissue, they are inadequate for thermally treated material due to the degradation of protein epitopes (Hird, Goodier, & Hill, 2003; Rodriguez, García, González, Hernández, & Martín, 2005). This limitation is minimized for DNA-based methods because the deoxyribonucleic acid helix is more stable than protein molecules. Therefore, DNA analysis provides a valuable alternative to ELISA technique. Both conventional PCR (Mane, Mendiratta, Tiwari, & Bhilegaokar, 2011) and real-time PCR using TaqMan and SYBR GREEN probes (Camma, Di Domenico, & Monaco, 2012; Fajardo et al., 2008a, 2008b; Kesmen, Gulluce, Sahin, & Yetim, 2009; Martín et al., 2008; Martín et al., 2010;

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Table 1
Composition of the reference material and source of origin.

Symbol used in the publication	Composition/mode of preparation	Symbol used in the publication	Composition/mode of preparation
B	100% bovine meat-and-bone meal[a]	S-TM	Feed mixture containing porcine meal[b]
S	100% porcine meat-and-bone meal[a]	O-TM	Feed mixture containing ovine meal[b]
D	100% poultry meal	B-TM	Feed mixture containing bovine meal[b]
B-0.1	0.1% bovine meat-and-bone meal/Proper amount of bovine meal (B) in KN	D-TM	Feed mixture containing poultry meal[b]
S-0.1	0.1% porcine meat-and-bone meal/Proper amount of porcine meal (S) in KN	KN	Negative control – feed mixture without animal meal[b]
D-0.1	0.1% poultry meal/Proper amount of poultry meal (D) in KN	KN1	Negative control – feed mixture without animal meal[b]
PW	Porcine plasma[a]	KN2	Negative control – feed mixture without animal meal/maize[c]
MB	0.5% bovine meat meal[a]	O/K	Cat food[c] containing ovine and poultry meat
K	Casein[a]	O/B	Cat food containing ovine and bovine meat[c]
SRW	Whey 10%[a]	KPB	Bovine blood[a]
MZ	Milk replacer 10%[a]	KPS	Porcine blood[a]
H	Hemoglobin[a]	KPO	Ovine blood[a]
SW	Pork lard[a]	KPD	Chicken blood[a]
B1	0.09% bovine meat-and-bone meal, 49.955% poultry meal, 49.955% porcine meal/B, S and D meals mixed in proper proportions[a]	D1	0.08% poultry meal, 49.955% porcine meal, 49.955% bovine meal/B, S and D meals mixed in proper proportions[a]
B2	0.09% bovine meat-and-bone meal/99.91% porcine meal/B and S meals mixed in proper proportions[a]	D2	0.08% poultry meal/99.92% bovine meal/B and D meals mixed in proper proportions[a]
B3	0.09% bovine meat-and-bone meal/99.91% poultry meal/B and D meals mixed in proper proportions[a]	D3	0.08% poultry meal/99.92% porcine meat-and-bone meal/S and D meals mixed in proper proportions[a]
B4	0.09% bovine meal, 4% ovine meal, 10% porcine meal, 10% poultry meal/B, D, S and O-TM meals mixed in proper proportions[a]	D4	0.08% poultry meal, 4% ovine meal, 10% bovine meal, 10% porcine meal/B, D, S and O-TM meals and negative control (KN) mixed in proper proportions[a]
B5	0.09% bovine meal, 0.5% ovine meal, 0.5% porcine meal, 0.5% poultry meal/B, D, S and O-TM meals and negative control (NC) mixed in proper proportions[a]	D5	0.08% poultry meal, 0.5% ovine meal, 0.5% bovine meal, 0.5% porcine meal/B, D, S, and O-TM meals and negative control (KN) mixed in proper proportions[a]
B6	0.09% bovine meat-and-bone meal, 99.91% fish meal/Proper amount of bovine meal (B) in fish meal[a]	D6	0.08% poultry meal, 99.92% fish meal/Proper amount of poultry meal (D) in fish meal[a]
S1	0.09% porcine meat-and-bone meal, 49.955% poultry meal, 49.955% bovine meal/B, S and D meals mixed in proper proportions[a]	O1	0.09% ovine meal, 49.1% poultry meal, 49.1% porcine meal/TM-O, S and D meals mixed in proper proportions[a]
S2	0.09% porcine meat-and-bone meal, 99.91% bovine meal/B and S meals mixed in proper proportions[a]	O2	0.09% ovine meal, 98.2% porcine meal/TM-O and S meals mixed in proper proportions[a]
S3	0.09% porcine meat-and-bone meal, 99.91% poultry meal/S and D meals mixed in proper proportions[a]	O3	0.09% ovine meal, 98.2% poultry meal/TM-O and D meals mixed in proper proportions[a]
S4	0.09% porcine meat-and-bone meal, 4% ovine meal, 10% bovine meal, 10% poultry meal/B, D, S and O-TM meals and negative control (NC) mixed in proper proportions[a]	O4	0.09% ovine meal, 10% bovine meal, 10% porcine meal, 10% poultry meal)/B, D, S and O-TM meals mixed in proper proportions[a]
S5	0.09% porcine meat-and-bone meal, 0.5% ovine meal, 0.5% bovine meal, 0.5% poultry meal/B, D, S and O-TM meals and negative control (KN) mixed in proper proportions[a]	O5	0.09% ovine meal, 0.5% bovine meal, 0.5% porcine meal, 0.5% poultry meal/B, D, S, and O-TM meals and negative control (KN) mixed in proper proportions[a]
S6	0.09% porcine meat-and-bone meal, 99.92% fish meal/Proper amount of bovine meal (B) in fish meal[a]	O6	0.09% ovine meal, 99.91% fish meal/Proper amount of ovine meal (B) in fish meal[a]
		O7	0.09% ovine meal, 98.2% bovine meal/TM-O and B meals mixed in proper proportions[a]
		O8	0.09% ovine meal, 49.1% poultry meal, 49.1% bovine meal/TM-O, B and D meals mixed in proper proportions[a]
		O9	0.09% ovine meal, 49.1% porcine meal, 49.1% bovine meal/TM-O, B and S meals mixed in proper proportions[a]

[a] Commercial sample from IZ-PIB resources.
[b] Mixture from the International Test "Protein in animal feed PCR" Veterinary Laboratories Agency (VLA).
[c] Commercial samples purchased from a shop.

Pegels et al., 2012; Prado et al., 2007) have become most relevant. The PCR method enables the detection of trace amounts of animal tissues in amounts as small as 0.1% (Cawthraw et al., 2009; Fumière et al., 2009; Yancy et al., 2009). Many authors report using universal primers with restriction enzymes (PCR-RFLP) (Kumar, Singh, Karabasanavar, Singh, & Umapathi, 2012; Stamoulis, Stamatis, Sarafidou, & Mamuris, 2010), which makes it possible to identify the taxonomic group and then the species. Multiplex PCR, which enables the identification of several components at the same time, is also widely used (Chi, 2013; Dalmasso et al., 2004; Ghovvati, Nassiri, Mirhoseini, Heravi, & Javadmanesh, 2009; Ioja-Boldura, Popescu, Bruznican, & Hutu, 2011; Koppel, Ruf, & Rentsch, 2011; Şakalar & Mustafa, 2011). Although multiplex PCR reduces the unit cost of an assay and saves time, its application and standardization in species identification is hindered by the relatively low sensitivity of the test and the uneven amplification efficiency of different primers (Ballin, Vogensen, & Karlsson, 2009; Walker et al., 2003), which may produce false negative results (Chi, 2013; Walker et al., 2003).

The current study used mtDNA because it is found in considerable amounts (millions of copies) in every cell, which positively affects PCR sensitivity. Another advantage of mitochondrial DNA are the adequately studied and easily available GenBank sequences, which can be used to develop methods based on PCR and real time PCR (Dalmasso et al., 2004; Krcmár & Rencová, 2003; Prado, Casqueiro, Iglesias, Cepeda, & Barros-Velazquez, 2004). By