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Cocoa Husks Feeding Improves Some Indices of Health and Welfare of Pigs

D. Magistrelli Department of Agricultural and Environmental Sciences, University of Milan, via G. Celoria, 2 – 20133 Milan, Italy

L. Malagutti Department of Agricultural and Environmental Sciences, University of Milan, via G. Celoria, 2 – 20133 Milan, Italy

F. Rosi Department of Agricultural and Environmental Sciences, University of Milan, via G. Celoria, 2 – 20133 Milan, Italy

ABSTRACT

The study evaluated the effects of dietary inclusion of cocoa husks, as source of polyphenols, on haematological indicators of animal health and welfare. Eight pigs were divided into two groups: CTRL fed a conventional cereal-based diet, and COCOA fed a diet obtained by substitution of 10% of the control diet with cocoa husks. After 6 weeks, blood was analyzed for some indices of health and welfare. Cocoa husks increased HDL cholesterol and antithrombin III, and decreased haptoglobin concentration. In humans, these results are associated with reduction of cardiovascular risk. COCOA pigs showed higher concentrations of immunoglobulins than CTRL pigs. High levels of antibodies may improve resistance to pathogen infections and, in humans, are associated to reduction of cardiovascular risk. Cocoa husks feeding may improve health and welfare of pigs. In particular, considering pigs as models for humans, cocoa husks may help reduce the risk of cardiovascular diseases.

KEYWORDS animal welfare, cardiovascular risk, cocoa husks, health, pigs, polyphenols

INTRODUCTION

According to their nutritional composition some wastes from crops and agro-industry may have a potential for use in human or animal nutrition (Ulloa et al., 2004). Cocoa husks are examples of such wastes. Cocoa husks are the integuments of the beans of Theobroma cacao L., a plant tree originated in the rain forests of America, whose culture has extended to equatorial areas of Africa and Asia (Lecumberri et al., 2007a). Cocoa beans are used worldwide for chocolate production. After the harvest, cocoa beans are dried, roasted, packaged and sent to industrialized countries where the seeds are shelled and husks are removed. The shelled seeds are used for the production of chocolate, while the husks constitute a by-product, which is partially used to produce feedstuffs, fertilizers or food products (McShea et al., 2008).

Despite having a high content of insoluble fibre, cocoa husks contain appreciable amounts of soluble dietary fibre, proteins and lipids (Lecumberri et al., 2007a). Moreover, cocoa husks are a rich source of antioxidants, in particular polyphenols (Lecumberri et al., 2007a; Ramiro-Puig & Castell, 2009). According to their antioxidant activity, polyphenols can limit the production of reactive oxygen species (ROS), including free radicals, which represent a risk of degenerative disease (Ramiro-Puig et al., 2007a). Several authors report that cocoa polyphenols have several effects that may promote health (Matsui et al., 2005; Cooper et al., 2008; McShea et al., 2008; Ramiro-Puig & Castell, 2009; Khan et al., 2014).

The use of cocoa husks in pig nutrition was tested by some authors (Migdal et al., 2000; Magistrelli et al., 2012), but the possible effects of cocoa polyphenols on animal health and welfare were not considered.

Farm animal health and welfare can be assessed by measuring different haematological indicators (Magistrelli et al., 2013). The most frequent used parameter for the evaluation of animal health and welfare is blood cortisol, whose level is known to increase under stressful conditions (Matteri et al., 2000). Cortisol secretion from the adrenal cortex deeply influences metabolism, by stimulating the liver to convert fat and protein to intermediate metabolites that are ultimately converted to glucose (Matteri et al., 2000). Unfortunately, plasma cortisol may vary according to multiple factors, such as circadian rhythm, season and photoperiod, dietary composition and even episodic secretion from the adrenal gland (Barnett et al., 1981a; Ladewig & Smidt, 1989; Ruis et al., 1997). In addition, blood sampling itself causes a rise in cortisol concentration, so the withdrawal must be done as carefully and as rapidly as possible, in order to avoid restraint stress (Magnusson et al., 1998; Marchant-Forde et al., 2012). For this multiple variability of plasma cortisol, other parameters, such as a group of plasma proteins called acute phase proteins (APP), are usually used in the evaluation of animal health and welfare, together with cortisol. Plasmatic concentration of some APP, such as haptoglobin, ceruloplasmin and fibrinogen, rapidly increase (positive APP), whereas others, such as albumin and antithrombin III, decrease (negative APP), in animals subjected to different challenges, such as lesions, pathogen infections, stressful events and cardiovascular diseases (Knura-Deszczka et al., 2002; Chen et al., 2003; Geers et al., 2003; Saco et al., 2003; Murata et al., 2004; Petersen et al., 2004; Eryd et al., 2011). Positive APP are considered to be non-specific innate immune components synthesized by the liver to restore homeostasis, after the animal has been challenged (Murata et al., 2004). As consequence of positive APP secretion, the liver reduces the synthesis of negative APP to provide amino acids for the production of positive APP (Ritchie et al., 1999).

Animal health and welfare can be also investigated by the analysis of blood levels of antibodies. In addition of being the protagonists of humoral immunity, immunoglobulins are also indicators of poor animal welfare. Stress, in fact, negatively influences host immunity (Morrow-Tesch et al., 1994; Rose & Lang, 2006). This assumption is supported by observations of increased disease occurrence in animals exposed to extreme stressful environments, as a possible consequence of a down-regulation of the immune response (Blecha, 2000).

In the light of this, a pilot study has been done with the aim of evaluating the effects of the inclusion of cocoa husks, as source of polyphenols, in the diet for fattening Italian heavy

pigs on some haematological indicators of animal health and welfare.

MATERIALS AND METHODS

Eight castrated male Duroc x Large White pigs (Sus scrofa domesticus) homogeneous for weight (135 ± 4.06 kg; mean ± SEM), age (8.5 months) and genetics (same father) were randomly divided into two groups: a control group (CTRL) fed a conventional pelleted diet, based on cereals, and a treatment group (COCOA) fed a diet obtained by substitution of 10% of the control diet with coarsely-ground cocoa husks (Icam S.p.a. - Lecco, Italy). The cocoa husks were accurately mixed with the pelleted feedstuff in a screw mixer, so that animals weren't allowed to select the ingredients. The CTRL diet consisted of 49.0% of maize, 30.0% of barley, 10.0% of wheat bran, 7.50% of soy, 1.80% of calcium carbonate, 0.50% of sodium chloride, 0.50% of dicalcium phosphate, 0.50% of vitamin/mineral supplement, 0.15% of L-Lys HCl and 0.05% of DL-Met. The CTRL diet was formulated using standard nutrient requirements of INRA (Ferrando & Blum, 1989). Pigs were fed twice a day (8:00 and 17:00). Leftovers were removed from the trough and weighed before the administration of the diets. Feeding and fresh water were ad libitum. Animals were allocated into individual cages with slatted floor, in order to remove faeces and urines and keep the animals clean. Cages were in the same room, under identical natural conditions for light (14.5 ± 0.75 h/d), temperature (27.1 ± 2.49 °C) and humidity (65.0 ± 4.65%). The trial was conducted during July and August, in the North of Italy (latitude 45° 30′ 00″ N, longitude 9° 02′ 00″ E). After the experiment, pigs were destined to slaughtering. All the animals used in the trial were reared for and slaughtered in accordance with European Union guidelines (Directive 2010/63/EU) and considered clinically healthy by the local sanitary authority, over the entire study period.

During the study period, samples of the experimental diets and the cocoa husks were collected for analysis of chemical composition.

Individual dry matter (DM) intake was recorded daily, body weight (BW) was recorded weekly.

After 6 weeks of dietary treatment, jugular vein blood samples were taken from all the pigs by venipuncture, before the first meal of the day, using a sterile single-sample 19 G needle (1.1 x 40 mm) (Venoject - Terumo Europe N.V. - Leuven, Belgium). The blood was collected into 10-mL plastic vacuum tubes containing 17.6 mg of K_2EDTA as anticoagulant (Venosafe - Terumo Europe N.V. - Leuven, Belgium). Immediately after the withdrawal, collected blood was centrifuged at 2000 x g, for 15 minutes, at 4 °C. The plasma obtained was stored at –20 °C, pending analysis.

Collected samples of diets and cocoa husks were ground through a 1 mm screen (Fritsch GmbH - Idar-Oberstein, Germany) and analyzed for dry matter (DM) by drying at 105 °C until constant weight in a forced air oven (method 945.15) (Association of Official Analytical Chemists [AOAC], 1995) and crude protein by the macro-Kjeldhal technique (method 984.13) (Association Of Official Analytical Chemists [AOAC], 1995), using a 2300 Kjeltec Analyzer Unit (FOSS - Hillerød, Denmark). Minerals were determined by incineration at 550 °C for 3 h in a muffle furnace (method 942.05) (Association Of Official Analytical Chemists [AOAC], 1995), ether extract by extraction with petroleum ether in a Tecator Soxtec 2050 apparatus (FOSS - Hillerød, Denmark) (method 920.29) (Association of Official Analytical Chemists [AOAC], 1995). Neutral detergent fibre was determined by the method of Mertens (2002) with addition of sodium sulphite and α-amylase to the neutral detergent solution; acid detergent fibre and acid detergent lignin were determined by the method of Van Soest et al. (1991) using the Ankom 200 fibre apparatus (ANKOM Technology Corp. - Fairport, NY, USA). Fibrous fractions are reported on a mineral-free basis. Diets were analyzed for the content of total polyphenols using the Prussian Blue method (Budini et al., 1980) and for gross energy using an IKA 4000 adiabatic bomb calorimeter (IKA Werke GmbH K.G. - Staufen, Germany). Analysis of the experimental diets and the cocoa husks is presented in Table 1.

Table 1 Analysis of the experimental diets and cocoa husks (n = 3).

Item	Unit	Diet		Cocoa husks
		CTRL	COCOA	
Dry matter (DM)	%	87.8	88.3	91.9
Crude protein	% DM	15.0	15.4	19.0
Minerals	% DM	5.44	5.73	8.22
Ether extract	% DM	3.60	4.53	12.5
Neutral detergent fibre	% DM	12.8	14.2	26.5
Acid detergent fibre	% DM	5.03	7.19	25.8
Acid detergent lignin	% DM	1.21	2.22	10.9
Gross energy	MJ/kg DM	18.1	18.3	19.9
Total polyphenols	g/kg DM	8.18	15.4	71.2

Plasma was analyzed for metabolic traits and acute phase proteins (APP) by commercially available kits. Glucose, triglycerides, total cholesterol (Giesse Diagnostics S.r.l. - Rome, Italy) and non-esterified fatty acids (NEFA) (Wako Chemicals GmbH - Neuss, Germany) were analyzed by enzymatic colorimetric methods. HDL cholesterol was determined by enzymatic colorimetric method, after precipitation of LDL and VLDL cholesterol by a polyanionic reagent (Giesse Diagnostics S.r.l. - Rome, Italy). LDL cholesterol was determined by two-step enzymatic colorimetric method (Thermo Fisher Scientific Inc. - Middletown, VA, USA). In the first step, HDL and VLDL cholesterol were removed via selective reactions with cholesterol esterase and cholesterol oxidase, coupled to catalase reduction of the peroxide by-product. In the second step, catalase was inhibited and the remaining cholesterol (LDL cholesterol) was measured enzymatically. Total protein was determined by a colorimetric method based on reaction with the biuret reagent (Giesse Diagnostics S.r.l. - Rome, Italy). Urea and creatinine were analyzed by kinetic methods (Giesse Diagnostics S.r.l. - Rome, Italy). Haptoglobin and ceruloplasmin (positive APP) and antithrombin III (negative APP) were determined by turbidimetric methods (Giesse Diagnostics S.r.l. - Rome, Italy). Fibrinogen (positive APP) was determined by a method based on clotting time, after conversion of fibrinogen into fibrin by a standardized thrombin solution (Giesse Diagnostics S.r.l. - Rome, Italy). Albumin (negative APP) was determined by a colorimetric method based on reaction with the bromocresol green reagent (Giesse Diagnostics S.r.l. - Rome, Italy). Plasma was analyzed for cortisol (DRG International Inc. - Mountainside, NJ, USA) and imunoglobulins (IgA, IgG and IgM) by ELISA (Bethyl Laboratories Inc. - Montgomery, TX, USA).

One-way ANOVA, considering dietary treatment as independent variable, was performed using SAS software version 8.01 (SAS Institute Inc. - Cary, NC, USA). Differences between groups were determined by Student's t-test, using $p \leq 0.05$ as the level of significance. When $0.05 < p < 0.10$ results are discussed as tendencies toward significance. Data are presented as mean value ± SEM.

RESULTS

Cocoa husks feeding reduced DM intake by 8% (2.06 ± 0.04 kg/d vs. 1.89 ± 0.03 kg/d for CTRL and COCOA group, respectively, $p < 0.001$). Despite this, final body weight was not different between the experimental groups (162 ± 6.83 vs. 159 ± 5.91, $p = 0.702$).

Data on plasma analysis are shown in Table 2. No difference was observed in the concentration of plasma glucose, NEFA, triglyceride, total cholesterol, total protein, urea and creatinine between the experimental groups. By contrast, cocoa husks

feeding increased HDL cholesterol in COCOA pigs, without affecting plasmatic concentration of LDL cholesterol (Table 2).

Table 2 Analysis of metabolic traits, cortisol, acute phase proteins and immunoglobulins in plasma of pigs fed a negative control diet (CTRL) and a 10% cocoa husks diet (COCOA). Data are given as means per treatment ± SEM, n = 4.

Item	Unit	Group		p-value
		CTRL	COCOA	
Metabolic traits				
Glucose	mmol/L	4.52 ± 0.65	4.81 ± 0.02	0.674
NEFA	mmol/L	0.58 ± 0.11	0.63 ± 0.03	0.675
Triglycerides	mg/dL	90.6 ± 19.9	94.5 ± 4.79	0.784
Total cholesterol	mg/dL	75.9 ± 7.56	84.6 ± 1.95	0.251
HDL cholesterol	mg/dL	25.9 ± 5.34	38.5 ± 1.40	0.047
LDL cholesterol	mg/dL	33.3 ± 5.55	37.7 ± 3.21	0.499
Total protein	g/L	53.6 ± 6.77	52.2 ± 1.94	0.854
Urea	mmol/L	5.68 ± 0.81	5.87 ± 0.47	0.838
Creatinine	mmol/L	0.18 ± 0.02	0.19 ± 0.01	0.857
Cortisol	nmol/L	102 ± 11.3	102 ± 7.09	0.956
Acute phase proteins				
Haptoglobin	mg/dL	83.3 ± 20.6	35.1 ± 1.46	0.058
Ceruloplasmin	mg/dL	18.4 ± 2.78	21.2 ± 1.99	0.448
Fibrinogen	g/L	1.15 ± 0.11	1.13 ± 0.06	0.893
Albumin	g/L	21.9 ± 2.89	21.1 ± 0.97	0.806
Antithrombin III	mg/dL	8.89 ± 1.51	15.0 ± 2.11	0.057
Immunoglobulins				
IgA	g/L	0.75 ± 0.15	1.51 ± 0.18	0.029
IgG	g/L	7.42 ± 0.71	17.1 ± 2.96	0.020
IgM	g/L	1.10 ± 0.37	2.05 ± 0.07	0.045

Plasma cortisol, ceruloplasmin, fibrinogen and albumin were not different between CTRL and COCOA pigs, but the analysis of APP showed a tendency towards a decrease of plasmatic concentration of haptoglobin and an increase of plasmatic concentration of antithrombin III in the pigs that received the diet containing cocoa husks (Table 2).

Pigs that consumed cocoa husks showed levels of plasma immunoglobulins that were double than those observed for CTRL pigs (Table 2).

DISCUSSION

In comparison to control pigs, feeding fattening pigs with a diet containing cocoa husks for 6 weeks reduced DM intake, without negatively affecting body growth. Similar results have been previously observed in rats fed cocoa (Ramiro-Puig et al., 2007a) or cocoa husks (Lecumberri et al., 2007b) for 3 weeks. This finding may have great economic significance. Cocoa husks are by-products of chocolate production and have a very low cost, so, even if an analysis of feeding costs has not been developed in the present study, one could easily speculate that the use of cocoa husks in pig nutrition can contribute to reduce the cost of the livestock production system. Anyway, performance studies should be conducted on a wider number of experimental subjects, due to the variability of growth parameters (Aaron & Hays, 2004). According to this, in the present study, the lack of differences in body weight between the experimental groups could be depended on the number of animals. Further studies developed on a broader range of pigs will verify the role of cocoa husks in reducing breeding costs.

Consumption of coarsely-ground cocoa husks for 6 weeks increased plasma HDL cholesterol level in fattening pigs. The intake of polyphenols has been shown to increase baseline plasma HDL cholesterol concentration in both human and animal studies (Araya et al., 2001; Baba et al., 2007). Although the mechanism by which cocoa polyphenolic compounds elevates plasma HDL cholesterol concentrations remains unclear (Khan et al., 2012), the effect of the administration of cocoa polyphenols on plasma cholesterol has great interest from a human point of view, since, in humans, increased HDL cholesterol is associated with reduced risk of ischemic stroke (Lindenstrom et al., 1994; Sacco et al., 2001).

Plasma cortisol level was not different between CTRL and COCOA pigs, whereas a tendency towards a decrease of haptoglobin and an increase of antithrombin III was observed in the plasma of pigs consuming cocoa husks. The pigs of the present study were kept under high environmental temperatures and in individual cages without tactile interactions with other pigs. This condition is considered to be potentially stressful (Barnett et al., 1981b; Ruis et al., 1997; Huynh et al., 2005). Results obtained from the analysis of plasma APP suggest a better coping with the environmental conditions for COCOA pigs than CTRL ones. This could have been promoted by cocoa polyphenols intake, which can affect liver metabolism, in order to help animal acclimation, when animals are subjected to hot conditions (Magistrelli et al., 2012).

In human medicine, positive APP (including haptoglobin, ceruloplasmin and fibrinogen) are usually referred to as inflammation-sensitive plasma proteins (ISP). These proteins increase the cholesterol-related incidence of cardiovascular diseases: when hypercholesterolemia is associated with high plasma levels of ISP, the risk of ischemic stroke and myocardial infarction increases (Yarnell et al., 1991; Engström et al., 2002; Tang et al., 2010). Antithrombin III is a negative APP synthesized by the liver, whose main role is inhibiting thrombin, thus limiting the formation of blood clots. Reduced levels of antithrombin III may favor blood coagulation, with increased risk of cardiovascular adverse events, such as thrombosis and pulmonary embolism (Marciniak et al., 1974). For this, low levels of plasma haptoglobin and high levels of plasma antithrombin III have positive effects on human health (Engström et al., 2002; Wang et al., 2011).

Plasma immunoglobulin levels were twice higher in cocoa husks-fed pigs, in comparison with the control ones. This result is in contrast to what previously observed in rats. Ramiro-Puig, Pérez-Cano, Ramírez-Santana, Castellote, Izquierdo-Pulido, Permanyer, Franch and Castell (2007b) reported that feeding rats a 10% cocoa-enriched diet for 3 weeks reduces blood levels of antibodies. Massot-Cladera, Franch, Castellote, Castell and Pérez-Cano (2013) also reported that feeding rats a diet containing 0.4% of cocoa polyphenols for 2 weeks reduces blood level of IgM, but has no effect on IgG. As stated above, the pigs of the present study were under potentially stressful conditions (Barnett et al., 1981b; Ruis et al., 1997; Huynh et al., 2005) that may have caused immunosuppression in control pigs (Morrow-Tesch et al., 1994), which, however, did not result in a pathological situation. In control pigs, plasma IgA and IgG levels were below the physiological range, whereas, in cocoa husks-fed pigs, all the classes of immunoglobulins were within the normal immuno range (Sigma-Aldrich Corp., 2009). According to this, cocoa husks may have a role in supporting the immune system, under stressful conditions. The analysis of plasma immunoglobulins is a useful tool to assess animal welfare (Morrow-Tesch et al., 1994; Blecha, 2000; Rose & Lang, 2006) and obtained results suggest a better coping with the environmental conditions for COCOA

pigs than CTRL ones. High levels of antibodies (within the normal immuno range) also have positive effects on health, by improving the resistance to pathogen infections (Ochsenbein et al., 1999). Moreover, in humans, a variety of situations, other than pathogen infections, can lead to a constant or transient deficiency in immunoglobulins. Thus, replacement of immunoglobulin levels by intravenous administration is used to treat a wide set of diseases, including cardiovascular diseases (Nimmerjahn & Ravetch, 2008; Udi & Yehuda, 2008).

CONCLUSION

Administration of cocoa husks to pigs can improve animal health and welfare. Consumption of polyphenols-rich products seems to help animals in coping with potentially stressful environmental conditions. Moreover, cocoa polyphenols intake may support the immune system, enhancing the resistance of the animals to pathogen infections, thereby reducing the use of antibiotics.

Considering pigs as models for humans, the present study confirms that consumption of foods rich in polyphenols may lower the risk of cardiovascular diseases (Estruch, 2012; Khan et al., 2014). In fact, this study shows that cocoa polyphenols intake positively affects some biomarkers of cardiovascular diseases. Pigs share many anatomical and physiological similarities with humans, and applications of pigs in biomedical research are numerous and ever growing, especially in cardiovascular research (Bähr & Wolf, 2012). According to this, obtained results highlight a potential role for cocoa husks in prevention and treatment of cardiovascular diseases, in addition to the medical therapies.

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