

## Consumption of brown onions (*Allium cepa* var. *cavalier* and var. *destiny*) moderately modulates blood lipids, haematological and haemostatic variables in healthy pigs

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Although garlic and onions have long been associated with putative cardiovascular health benefits, the effects of different commercially available onions and level of intake have not been studied. Therefore, the aim of the present study was to evaluate the potential health benefits of raw onions using the pig as a biomedical model. Twenty-five female (Large White × Landrace) pigs were used in a (2 × 2) + 1 factorial experiment. Pigs were fed a standard grower diet supplemented with 100 g tallow/kg with the addition of *Allium cepa* var. *cavalier* or var. *destiny* at 0, 10 or 25 g/MJ digestible energy for 6 weeks. Overall, the consumption of onions resulted in significant reductions in plasma triacylglycerol; however, the reductions were most pronounced in pigs fed *destiny* onions (–26%,  $P=0.042$ ). Total plasma cholesterol and LDL:HDL ratios were not significantly different. Onion supplementation, regardless of the variety, resulted in dose-dependent reductions in erythrocyte counts and Hb levels, while the white blood cell concentrations, particularly lymphocytes, were increased in pigs that consumed onions. Furthermore, indices of blood clotting were largely unaffected by onion consumption. In conclusion, dietary supplementation with raw brown onions has moderate lipid-modulating and immunostimulatory properties. However, daily onion intake > 25 g/MJ digestible energy could be detrimental to erythrocyte numbers.

### Onions: Health: Lipid metabolism: Pig

There is an increasing amount of consumer interest and research focused on various food products that offer a positive health benefit beyond basic nutrition ('functional foods'). Plants from the genus *Allium*, particularly onions (*A. cepa*) and garlic (*A. sativum*), have been consumed for their putative nutritional and health benefits for centuries. Although the health functionality of garlic has been reported extensively (Bordia & Verma, 1980; Ali & Thomson, 1995; Dorant *et al.* 1995; Smith & Yang, 2000; Liu & Yeh, 2001; Slowing *et al.* 2001), very little is known about the specific benefits of onion (Bordia & Verma, 1980; Ali & Thomson, 1995; Dorant *et al.* 1995; Ide *et al.* 1997; Smith & Yang, 2000; Slowing *et al.* 2001). Epidemiological studies have shown a correlation between diets rich in onion and reduced risk of stomach cancer in human subjects (You *et al.* 1989), as well as an inverse relationship with mortality from CHD in man (Hertog *et al.* 1993a,b). Compounds that have been implicated in providing a number of health-promoting attributes of onion include flavonoids, particularly the flavonol

quercetin and the organosulfur compounds such as cysteine sulfoxides (CSO) (Price & Rhodes, 1997). S compounds from garlic, and common to onion, have been shown to reduce plasma total cholesterol, LDL-cholesterol and triacylglycerol (TG) levels in human subjects and rodents (Bordia & Verma, 1980; Chi, 1982; Chi *et al.* 1982; Qureshi *et al.* 1983a,b; Slowing *et al.* 2001); flavonols such as quercetin have also been shown to have lipid-modulating properties (Bok *et al.* 2002; Glasser *et al.* 2002).

In the absence of clinical studies on the effects of raw onion consumption, the present study used a pig model to characterise the functional properties of two onion cultivars grown in different environments and agronomic conditions. The pig is a good animal model for human nutrition, because of the many similarities between pigs and man in the anatomy and physiology of the digestive (Book & Bustad, 1974; Swenson, 1977) and cardiovascular (Martin, 1964; Lumb, 1966) systems. Furthermore, the advantage of the pig in biomedical research is the similarity in the major lipoprotein subclass of LDL

physico-chemical characteristics, albeit that the levels of plasma lipids are lower in pigs than human subjects (Chapman & Goldstein, 1976).

## Materials and methods

### Onions

Two varieties of onions commercially grown in the Australian states of Tasmania (cool-temperate climate) and Queensland (sub-tropical climate) were chosen for the present study. Both varieties were brown onion. *A. cepa* var. *cavalier* was grown in Gatton, Queensland (approximately 152° E, 28° S), while *A. cepa* var. *destiny* was sourced from the Devonport region of Tasmania (approximately 145° E, 40° S). DM was determined in triplicate by drying homogenised onion samples to a constant weight in a force-draught oven at 105°C.

### Animals and handling

All procedures were approved by the Victorian Institute of Animal Science Animal Ethics Committee (Anonymous, 1997). Twenty-five female crossbred (Large White × Landrace) pigs (initial body weight (BW) 41.5 (SD 4.2) kg) were placed in individual pens, blocked according to initial live weight and allocated to one of five dietary treatments, in a randomised block design. The blocks were formed using the initial live weights of the pigs. Pigs were fed approximately 90–95% of *ad libitum* intake (1.67 MJ digestible energy (DE)/kg BW<sup>0.75</sup>) of a dry feed formulated to contain 16.7 MJ DE/kg and 100 g tallow/kg to simulate the saturated fatty acid content of a western human diet. The energy intake from fat was about 3.6 MJ or 21.5%. The experimental diet (Table 1) was formulated to be in excess of protein and lysine requirements for this class of pigs (Dunsha *et al.* 1993). The amino acid content relative to lysine was kept in excess of the amino acid balance proposed as ideal by the Standing Committee on Agriculture (1987). A vitamin and mineral premix was omitted from the diets as it contains antioxidants and

other compounds that could mask the effects exerted by the onion. Pigs had access to water at all times.

The five experimental treatments consisted of a control diet with no onion added, and a low (16 g/kg BW<sup>0.75</sup> or 10 g/MJ DE) and a high (40 g/kg BW<sup>0.75</sup> or 25 g/MJ DE) dose of onion for each variety of onion. The amount of onions fed to the pigs was set at levels that can be consumed by human subjects, after normalisation for the comparative differences in energy intake between pigs and human subjects. The low dose would be equivalent to about half an onion per d and the high dose equivalent to about one and a half large onions per d in human subjects. Onions were stored at 4°C, and diets were prepared daily to maintain stability of the active compounds found in onions. The loose outer epidermis of the onion was removed and the onions were then chopped in half and homogenised in a blender for approximately 2 min. The calculated amount of the homogenate was weighed for each pig and mixed together with the dry feed for 2 min until the feed reached uniform consistency. Diets were fed within 1 h of preparation, as the thiosulfates (the product of the breakdown of CSO when the onions are cut-up) are highly unstable. Pigs were weighed once per week and the amount of dry feed and onion adjusted according to BW. Feed refusals were collected and recorded daily.

### Blood collection and analysis

Blood samples were obtained from the jugular vein by venepuncture before feeding (fasted) on two sequential days after 4 and 6 weeks of treatment. Blood was also collected from each pig 3 h post-feeding (postprandial), since the absorption of dietary flavanols from onions in human blood reaches peak values between 0.7 and 2.9 h after feeding (Hollman *et al.* 1996, 1999; Aziz *et al.* 1998).

On the first sequential sampling day, blood was drawn into EDTA, clot activator and sodium citrate vacutainer tubes, which were dedicated for blood coagulation tests (platelet count, prothrombin time, activated partial prothrombin time and fibrinogen counts) and blood biochemistry assays (cholesterol, TG and glucose). Two blood smears were prepared immediately for each pig, using blood that contained EDTA as an anticoagulant, to determine the number of white blood cells, erythrocytes, eosinophils, segmented neutrophils, lymphocytes, monocytes and basophils. Blood samples were placed on ice and transported for analysis (IDEXX Laboratories Pty Ltd, Mount Waverley, Victoria, Australia). On the second day of sampling, three blood samples were collected in vacutainer tubes containing EDTA, heparin and without anticoagulant respectively. Half of the blood collected into the EDTA tube was immediately decanted after bleeding into a tube that contained 10 µM-indomethacin (final concentration) for 11-dehydro-thromboxane B<sub>2</sub> (TXB<sub>2</sub>) assay. Plasma was separated by centrifugation at 3000 rpm for 15 min and stored at -20°C until analysed. The rest of the blood collected on the second day was used to determine concentrations of plasma HDL-cholesterol (procedure no. 354L) and total cholesterol (procedure no. 401) using kit reagents purchased from Sigma (Sigma-Aldrich Pty Ltd, Sydney, Australia). To assess clotting capability of the

**Table 1.** Composition of experimental diets\*

Ingredients	g/kg
Wheat	679.0
Soyabean meal	91.4
Fish meal	45.7
Meat and bone meal	57.1
Blood meal	19.0
Tylan†	0.4
NaCl	1.8
Ca <sub>2</sub> PO <sub>4</sub>	4.3
L-Lys hydrochloride	0.9
DL-Met	0.1
L-Thr	0.2
Tallow	100.0

\* Diet was formulated to contain 16.7 MJ digestible energy, 177 g crude protein (N × 6.25) and 9.4 g available lysine/kg air-dry diet; raw onions (*Allium cepa*) were mixed with the dry feed at 10 or 25 g/MJ digestible energy.

† Tylosin phosphate 100 g/kg antibiotic (Elanco Animal Health, Macquarie Park, NSW, Australia).

blood, TXB<sub>2</sub>, a stable metabolite of thromboxane A<sub>2</sub> concentrations (catalogue no. 519501; Cayman Chemical Pty Ltd, Crows Nest, NSW, Australia) were measured.

#### Cysteine sulfoxide and quercetin analyses

The analysis of CSO in raw onion tissue was carried out with an HPLC methodology based on that of Yoo & Pike (1998), with the exception that the samples were frozen at  $-70^{\circ}\text{C}$  before extraction and 10 mM-hydroxylamine was added to the extraction solvent (i.e. 800 ml ethanol/l with 10 mM-hydroxylamine). The level of acetic acid in the mobile phase was increased to 6 ml/l. The mean concentration of CSO was calculated from analysis of ten onions of each variety. For quercetin analysis, freeze-dried onion powder from at least three separate onions per treatment was hydrolysed in HCl at  $90^{\circ}\text{C}$  for 2 h, and total quercetin aglycone was assayed using HPLC according to the method described by Hertog *et al.* (1992).

#### Statistical analyses

Data was analysed by ANOVA using GENSTAT for Windows, version 4.1 (Payne *et al.* 1993). The results are presented as mean values of the fasting samples and 3 h postprandial measurements taken at weeks 4 and 6. Each mean value, as well as growth rate and average dry feed intake, was initially analysed using a randomised block design that included the types of onion and two doses (10 and 25 g onions/MJ DE) with the control (no onion) added. These initial results indicated that the blood measurements could usefully be divided into two groups. These were: (1) all blood measurements other than lipid biochemistry measurements, where there was no evidence of onion-type effects ( $P > 0.05$ ); (2) lipid biochemistry measurements, where there was often evidence of onion-type effects ( $P < 0.05$ ).

For lipid biochemistry measurements (Table 2), with the exception of cholesterol, there was no evidence of any

difference between the 10 and 25 g onions/MJ DE doses ( $P > 0.01$ ). The results are thus tabulated as the means of each type of onion as well as the mean of no onion. For the haematology measurements (Table 3) the main effects of two onion doses (0, 10 and 25 g onions/MJ DE) are tabulated together with results of hypothesis tests for the overall dose response and the linear component of this response. A comparison of the onion type within 10 and 25 g onions/MJ DE dose levels is also presented. In every analysis, a residual was used that allowed for the effect of all five treatments, as well as a blocking effect. Results showing the interactions between bleeding time and week-to-week variation as well as effects of dietary onions on oxidative capacity of serum are presented elsewhere (NK Gabler, E Ostrowska, SJ Sterling, BG Tatham, RB Jones, DR Eagling, M Jois and FR Dunshea, unpublished results).

#### Results

The DM contents of the onions used in this study were 97.5 and 127.5 g/kg for *cavalier* and *destiny* varieties respectively. Total CSO was 23% higher in *destiny* than *cavalier* onions, although this difference was not significant ( $P = 0.16$ , Table 4). Of the three major CSO found in onions, *S*-methyl-CSO and *S*-propyl-CSO were significantly higher ( $P < 0.05$ ) in *destiny* compared with *cavalier* onions. Indeed, for the *cavalier* variety, *S*-propyl-CSO was undetectable. *S*-propenyl-CSO was the major CSO found in both onion varieties and there was no difference in *S*-propenyl-CSO levels between the two varieties. The variety *destiny* had a 38% higher quercetin aglycone content than *cavalier* ( $P < 0.05$ , Table 4).

All pigs offered onions remained in good health throughout the experimental period. No signs of toxicity were observed and onion consumption had no effect on growth rate (830 v. 860 g/d for control and onion-supplemented pigs respectively,  $P = 0.49$ ) or feed intake (1816 v. 1807 g DM/d respectively,  $P = 0.90$ ). Overall, dietary onion

**Table 2.** The effect of two onion (*Allium cepa*) varieties on blood biochemistry\* (Mean values)

Plasma concentration	Control	Onion variety		SED†	Statistical significance of effect	
		<i>Cavalier</i>	<i>Destiny</i>		Overall‡	Onion§
Cholesterol (mmol/l)	2.71	2.52	2.44	0.13	0.15	0.073
Glucose (mmol/l)	5.70	5.88	5.98	0.22	0.46	0.26
LDL (mmol/l)  ¶	1.65	1.56	1.51	0.10	0.21	0.52
HDL (mmol/l)	1.09	0.95	0.97	0.06	0.35	0.032
LDL:HDL	1.54	1.66	1.57	0.08	0.32	0.15
TG (mmol/l)	0.68	0.63	0.50	0.07	0.042	0.12
TG after (mmol/l)**	0.78	0.79	0.60	0.07	0.0067	0.18
TG before (mmol/l)††	0.57	0.48	0.41	0.09	0.25	0.16

TG, triacylglycerol.

\* For details of diets and procedures, see Table 1 and p. 212.

† SED for control v. *cavalier* or *destiny*; for *cavalier* v. *destiny* and control v. pooled onion, multiply the SED by 0.817 and 0.888 respectively.

‡ *P* value for overall comparison of control v. *cavalier* v. *destiny*.

§ *P* value for comparison of control v. pooled onion.

|| Mean of values taken in fasting and non-fasting states after 4 and 6 weeks of feeding (results were pooled across doses of each onion variety).

¶ VLDL, intermediate-density lipoprotein and LDL concentration calculated by subtracting the HDL from total cholesterol concentration.

\*\* Mean of values taken from non-fasting pigs after 4 and 6 weeks of onion feeding (results were pooled across doses of each onion variety).

†† Mean of values taken from fasting pigs after 4 and 6 weeks of onion feeding (results were pooled across doses of each onion variety).

**Table 3.** The effect of onion (*Allium cepa*) dose on haematology and measures of immunity\* (Mean values)†

	Dose (g/MJ DE)			SED‡	Statistical significance of effect	
	0	10	25		Linear§	Overall
Erythrocytes (10 <sup>12</sup> /l)	7.78	7.13	6.50	0.29	0.00040	0.0015
Hb (g/l)	134	131	124	4	0.046	0.13
MCV (fl)	53.6	55.9	59.8	2.0	0.0045	0.016
MCHC (pg)	17.3	18.1	19.2	0.8	0.028	0.083
PCV (l/l)	0.403	0.398	0.388	0.013	0.20	0.42
Basophils (10 <sup>9</sup> /l)	0.38	0.34	0.38	0.06	0.82	0.80
Eosinophils (10 <sup>9</sup> /l)	0.46	0.62	0.44	0.09	0.24	0.034
Lymphocyte activation¶	1.9	1.7	1.5	0.1	0.00081	0.0029
Lymphocytes (10 <sup>9</sup> /l)	8.5	11.3	9.7	1.0	0.87	0.020
Monocytes (10 <sup>9</sup> /l)	1.36	1.39	1.19	0.17	0.21	0.35
Neutrophil:lymphocyte ratio	0.61	0.43	0.49	0.08	0.39	0.068
Segmented neutrophils (10 <sup>9</sup> /l)	5.1	4.6	4.4	0.4	0.096	0.23
WBC (10 <sup>9</sup> /l)	15.7	18.3	16.1	1.1	0.46	0.016

DE, digestible energy; MCV, mean corpuscular volume; MCHC, mean corpuscular Hb concentration; PCV, packed cell volume; WBC, white blood cells.

\* For details of diets and procedures, see Table 1 and p. 212.

† Mean of values taken in fasting and non-fasting states at 4 and 6 weeks of feeding (for each dose, results were pooled across both onion varieties).

‡ SED for control v. 10 or 25 g onion/MJ DE; for a comparison between 10 or 25 g onion/MJ DE, multiply the SED by 0.817.

§ P value for linear effect of dose of onion.

|| P value for overall comparison of control v. 10 or 25 g onion/MJ DE.

¶ Lymphocyte activation measured as slight (score 1), moderate (score 2) and high (score 3).

supplementation tended to decrease circulating cholesterol (−9%,  $P=0.073$ , Table 2). However, there was an interaction of onion dose × type of onion, such that mean fasting and postprandial plasma cholesterol measures were decreased by 14% at the low, but not at the high, intake of *cavalier* onions, while plasma cholesterol was decreased by 6 and 14% ( $P=0.009$ ) at the low and the high intakes of *destiny* onions respectively (mmol/l: control 2.71, low dose of *cavalier* 2.29, high dose of *cavalier* 2.73, low dose of *destiny* 2.55, high dose of *destiny* 2.33; SED 0.15).

There was no apparent difference in plasma cholesterol concentration between fasting and non-fasting states (2.53 v. 2.52 mmol/l respectively,  $P=0.71$ ). The mean HDL concentration was significantly lower ( $P=0.032$ ) in pigs that consumed diets supplemented with onions, while LDL concentration was unaffected ( $P=0.52$ ). The LDL:HDL ratio was unchanged by onion consumption (Table 2).

While overall plasma TG concentrations were not significantly affected by dietary onion consumption (Table 2), there was a significant ( $P<0.001$ ) interaction in the response to different onion types and the nutritional

state of the pigs. Plasma TG concentrations were reduced in the 3 h postprandial measurements for pigs fed *destiny* but not *cavalier* onions (0.78 v. 0.79 and 0.60 mmol/l for control, *cavalier* and *destiny* respectively,  $P=0.0067$ ). Conversely, the TG concentrations were not significantly different in fasting animals fed the different diets. There was no difference in the plasma glucose concentrations between pigs fed the different onion varieties or between onion-fed and control pigs (Table 2).

The plasma erythrocyte number was reduced linearly in response to the increased amount of onion in the diet ( $P=0.0004$ , Table 3). Consequently, mean Hb concentration was decreased in a dose-dependent manner ( $P=0.046$ ); however, the overall comparison of control with low or high onion doses was not significantly different ( $P=0.13$ ). The mean erythrocyte cell volume was higher ( $P=0.016$ ) in pigs that consumed onions compared with the control group.

Pigs that consumed low doses of onion, regardless of the onion variety, had higher eosinophil, lymphocyte and total white blood cell numbers ( $P=0.034$ ,  $P=0.020$  and

**Table 4.** Cysteine sulfoxide (CSO) and quercetin contents (mg/g fresh weight for *Allium cepa* var. *cavalier* and *destiny*)\* (Mean values and standard deviations for analysis of three onions picked at random)

Onion variety...	<i>Cavalier</i>		<i>Destiny</i>		Statistical significance of effect: P
	Mean	SD	Mean	SD	
S-methyl-CSO	0.17	0.05	0.27	0.06	0.049
S-propenyl-CSO	0.71	0.17	0.72	0.16	0.92
S-propyl-CSO	ND†		0.15	0.10	–
Total CSO	0.88	0.22	1.14	0.23	0.16
Quercetin	0.22	0.02	0.48	0.04	0.013

ND, not detected.

\* For details of procedures, see p. 212.

† Detection limit about 0.07 mg/g; the DM contents for *cavalier* and *destiny* onions were 97.5 and 127.5 g/kg respectively.



**Table 5.** The effect of onion (*Allium cepa*) dose on coagulation profile of pig blood\* (Mean values)†

	Onion variety			SED‡	Statistical significance of effect
	Control	<i>Cavalier</i>	<i>Destiny</i>		Overall§
APTT (s)	22.3	22.1	23.7	1.1	0.10
Fibrinogen (g/l)	1.02	0.95	0.98	0.08	0.68
PT (s)	13.9	14.1	14.4	0.2	0.15
TXB <sub>2</sub> (pg/ml)	19.8	23.2	19.6	1.9	0.030
Platelets (10 <sup>9</sup> /l)	415	391	380	30	0.66

APTT, activated partial prothrombin time; PT, prothrombin time; TXB<sub>2</sub>, thromboxane B<sub>2</sub>.

\* For details of diets and procedures, see Table 1 and p. 212.

† Mean of values taken in fasting and non-fasting states at 4 and 6 weeks of feeding.

‡ SED for control v. *cavalier* or *destiny* onion; for a comparison between onion varieties, multiply the SED by 0.817.

§ P value for overall comparison of control v. *cavalier* or *destiny* onion.

$P=0.016$  respectively) compared with the control pigs (Table 3). The lymphocyte activation decreased as the dose of onion increased, resulting in a significant linear effect ( $P=0.00081$ ). For all haematology measures there was no difference in the response between the two onion varieties (Table 3).

The effect of onion supplementation on measures of blood clotting resulted in a small, but non-significant ( $P=0.15$ ) increase in prothrombin time in pigs fed onions, but no effect on activated partial prothrombin time (Table 5). The blood fibrinogen concentration and platelet number were unaffected by onion consumption. The measures of blood coagulation were not affected by onion variety ( $P>0.10$ ) with the exception of the mean plasma TXB<sub>2</sub> concentration, which was significantly higher ( $P=0.030$ ) in pigs fed *cavalier* but not *destiny* onions compared with the control pigs.

## Discussion

In general, consumption of raw brown onions was found to moderately modulate blood lipids and haematological variables in healthy pigs, but the responses varied between the two onion varieties and between doses of onions. In addition, differences in the response to consumption of onion were also evident between fasted and non-fasted states. For example, the postprandial TG measurements in pigs fed *destiny* onions were approximately 23% lower than in control pigs or the pigs fed *cavalier* onions, while fasting measurements were not significantly affected by onion consumption (Table 2). Postprandial measurements of TG levels are useful, as they might reveal a state of fat intolerance that cannot be detected by the simple measurement of fasting plasma TG (Karpe, 1999). Therefore, these results suggest that the consumption of *destiny* onions, at both doses investigated, resulted in beneficial changes in plasma postprandial TG concentrations. This may lead to reduced risk of CHD and atherogenesis (Zilversmit, 1979; Patsch *et al.* 1992).

Unlike TG, there was no significant difference between fasting and postprandial measurement of plasma cholesterol, which is in agreement with results from studies with human subjects (Cohn *et al.* 1988). However, onion consumption resulted in a 9% lower plasma total cholesterol concentration (mean fasting and postprandial

measurements) compared with the control group. There was a more pronounced effect in pigs fed *destiny* onions, where a linear decrease in plasma cholesterol in response to dose was observed. The HDL-cholesterol fractions were reduced in response to onion supplementation; however, the LDL:HDL ratio was unaffected. This suggests that the removal of cholesterol from the circulation was not enhanced, and it is likely that the cholesterol-lowering action of *destiny* onions was due to a reduction in cholesterol synthesis.

Despite similarities between pigs and human subjects, there are some differences in lipoprotein metabolism that must be considered. Pig blood contains lower cholesteryl ester transfer protein activity and cholesteryl ester formation than human subjects (Chapman, 1986) and treatments that affect lipoprotein metabolism might have a different impact on the levels of HDL in pigs than in human subjects (Knipping *et al.* 1987). Second, LDL-ApoB originates from VLDL catabolism in human subjects, whereas it is synthesised *de novo* in pigs (Birchbauer *et al.* 1992). Therefore, the extrapolation of these data to adult human subjects should be treated with caution.

While the mechanisms responsible for the lowering of lipid due to onion consumption are not clear, it has been suggested that *Allium* compounds (possibly CSO and thio-sulfinates) decrease synthesis or increase excretion of lipids through the intestinal tract (Ali *et al.* 2000; Bok *et al.* 2002). Various garlic extracts have also been shown to decrease the activities of NADPH-producing enzymes and of fatty acid synthetase when fed to rats (Chi, 1982; Chi *et al.* 1982), chickens (Qureshi *et al.* 1983b) and pigs (Chi, 1982; Chi *et al.* 1982; Qureshi *et al.* 1983a, 1987). However, garlic extracts are generally more potent than onion extracts. This is due to differences in the levels and types of S compounds, which are present in far lower concentrations in onions than in garlic. For example, thiosulfinates are proposed to exist in garlic at 100 times the concentration found in onions (Mandon *et al.* 2000).

In addition to the lipid-modulating effects of onion, changes in blood cell counts were also evident. Raw onion consumption reduced both erythrocyte numbers and Hb concentrations (Table 3), although clinical anaemia was not evident and the measures were within the normal range for pigs (Pond & Houpt, 1978). Reductions in

haematocritic measurements have also been observed in placebo-controlled studies with human subjects using encapsulated onion (Kalus *et al.* 2000; Mayer *et al.* 2001). Onion consumption has been shown to induce acute haemolytic anaemia in sheep (Kirk & Bulgin, 1979), horses (Pierce *et al.* 1972), cattle (Rae, 1999; van der Kolk, 2000), dogs (Spice, 1976) and cats (Kobayashi, 1981), largely due to the presence of onion disulfides: these generate H<sub>2</sub>O<sub>2</sub> in the presence of Hb and glutathione S-transferase within intact erythrocytes (Munday *et al.* 2003). It has been suggested that the resulting haemolysis is of the oxidative type and occurred due to removal of damaged erythrocytes by cells of the reticulo-endothelial system (Munday *et al.* 2003). Although the erythrocyte concentrations were decreased linearly by onion consumption in the present study, there was a compensatory increase in cell volume and the amount of Hb per cell, which implies an increased O<sub>2</sub>-carrying capacity per erythrocyte. The excessive consumption of onions should be avoided, especially by individuals with anaemia or those whose erythrocytes are unusually vulnerable to oxidative damage (Munday *et al.* 2003).

Reductions in haematocritic measures appeared to stimulate an immune response in the pigs as evident by the increase in white blood cell numbers, mainly due to increased lymphocyte concentrations in pigs that consumed onions (Table 3). Moreover, blood from pigs fed onion-supplemented diets exhibited decreased lymphocyte activation, which is often associated with reduced cholesterol accumulation and the reduction in formation of atherosclerotic plaques (Hansson, 1994).

Surprisingly, the measures of clotting mechanisms such as prothrombin time, activated partial prothrombin time, platelet count and fibrinogen level were largely unaffected by onion consumption (Table 5). Other studies that investigated the effects of garlic consumption have shown increased bleeding and clotting times (Gadkari & Joshi, 1991). Similarly, raw juice from shoots of Welsh onion (*Allium fistulosum*) fed to rats for 4 weeks at a dose of 2 g/kg per d significantly increased tail bleeding time and lowered systolic blood pressure (Chen *et al.* 2000). However, as these authors pointed out, the green shoots of the onion are more potent than the bulb.

Plasma TXB<sub>2</sub> was significantly increased in pigs fed *cavalier* but not *destiny* onions (Table 5). Previous observations have shown that raw garlic extracts significantly decrease rabbit serum TXB<sub>2</sub> levels, while onion extracts were found to be ineffective (Ali *et al.* 1999). Similarly, in human subjects, consumption of 70 g raw onion/d, which is equivalent to the low dose of onion administered to the pigs in the present study, had no effect on serum TXB<sub>2</sub> levels after 7 d of consumption (Srivastava, 1989). The differences across studies are possibly related to the differences in the level and type of organosulfur compounds in garlic and onion, and these are known to mediate eicosanoid metabolism (Ali *et al.* 2000).

Many of the biochemical effects of dietary onion consumption have been largely ascribed to CSO, which are abundant in intact onion bulbs. Garlic bulbs contain mainly allyl-CSO and to lesser extent *S*-methyl-CSO, whereas *S*-propenyl-CSO is the predominant *S* compound

in onion (Keusgen *et al.* 2002). Analysis of CSO in the onions used in the present study showed that there was little difference in total CSO between the two onion varieties, but *destiny* had significantly higher levels of *S*-methyl-CSO and *S*-propyl-CSO than *cavalier* (Table 4). Since *S*-methyl-L-CSO was found to be one of the factors responsible for suppressing hypercholesterolaemia in the hepatoma-bearing rat (Komatsu *et al.* 1998), it is possible that the differences between responses to different onion treatments in the present study are due to these compounds. Another possibility for the plasma lipid and haematological changes could result from thiosulfinates, which are the products of enzymatic degradation of sulfoxide compounds upon ingestion by human subjects and animals (Earl & Smith, 1983). Finally, it is likely that changes observed in the present study are due to a combination of *S* and flavonol compounds found in onions, of which flavonols have also been shown to be biologically active (Taucher *et al.* 1996).

Flavonols have been shown to affect cholesterol synthesis and reduce thrombotic tendencies (O'Reilly *et al.* 2000; Giugliano, 2000). Quercetin is the dominant flavonol in onions, with levels up to 490 mg/kg fresh weight (Hollman & Arts, 1994). The quercetin contents of both varieties of onions fed to the pigs in the present study were in the range reported in literature, but was significantly higher in the *destiny* onions. Therefore, the variation in quercetin levels may also be responsible for some of the health benefits observed in the present study. Further work needs to be conducted to elucidate the effect of different CSO and flavonoid compounds in onions to identify which are responsible for the observed biological responses in the pig.

## Conclusion

The present study has shown that onions have functional properties with the ability to modify lipid metabolism and stimulate the immune system. However, the differences in the responses observed in the present study demonstrate the complex interaction between the onion dose and type of onion, which may be due to the differences in the amount of active compounds. The identification of specific compounds in different onion cultivars and agronomic practices would lead to a better understanding of the physiological responses to onion consumption. This would aid the development of onion production systems that provide an increased health benefit and the development of guidelines for the consumption of these compounds.

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