Effects of a Dietary Chitosan-Alginate-Fe(II) Complex on Meat Quality of Pig Longissimus Muscle during Ageing

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ABSTRACT : The current study was conducted to investigate the effects of dietary chitosan-alginate-Fe(II) complex (CAFC) supplementation on carcass and meat qualities of pig *m. longissimus* during chiller ageing. One hundred and twenty-two LYD (Landrace×Yorkshire×Duroc) pigs were sampled from an industrial population. Seventy-four pigs (32 gilts and 42 barrows) were administered 3 ml of dietary supplementation of CAFC per day from 25 to 70 days of age, while the remaining 48 pigs (20 gilts and 28 barrows) were fed the same commercial feeding regime without the supplementation. For assessing the dietary effects on pH, objective meat color, cooking loss, water-holding capacity (WHC), thiobarbituric acid reactive substances (TBARS), volatile basic nitrogen (VBN) and fatty acid composition during ageing, 20 barrows (10 of each treatment) were randomly sampled, and aged for 3, 7, 12, 16, 20 and 25 days in a 1°C chiller. The results showed that CAFC-fed pigs required approximately 10 fewer feeding days than the control group. Furthermore, the treatment resulted in greatly higher carcass grade whereby the grade A was increased by approximately 35% and 7% for gilts and barrows, respectively. The treatment had no significant effect ($p>0.05$) on pH, meat color and WHC during ageing. On the other hand, the CAFC-fed pigs showed significantly ($p<0.05$) lower TBARS values from 20 days of storage. In addition, the sum of unsaturated fatty acids for the treated group was significantly ($p<0.05$) higher than that for the control group after the storage time. This implied that CAFC supplementation could reduce the formation of free radicals in fatty acids (i.e., lipid oxidation). The treatment also significantly ($p<0.05$) retarded VBN formation during ageing, indicating a significant reduction in protein degradation. However, as there was no difference in pH between the two groups, the result raised a possibility that antibacterial activity of the CAFC alone could cause reduction in the formation of TBARS and VBN. In this regard, although the treatment effectively slowed down the formation of TBARS and TBA during chiller ageing, it was not resolved whether that was associated with the direct effect of the antioxidant function of chitosan and/or alginate, or a consequence of their antibacterial functions. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 414-419)***

Key Words : Pork Quality, Chitosan-Alginate-Fe(II) Complex, Fatty Acids, Ageing

INTRODUCTION

The most significant defect in pork quality control during chiller ageing is lipid oxidation (Ulu. 2004). as the undesirable process has a significant detrimental effect on meat flavor (Wood et al., 2003) and colour (Faustman et al., 1992). Extensive efforts have been made to overcome this problem through diatary supplementation of vitamin E. selenium and/or conjugated linoleic acid, and with some success (Monahan et al., 1992a,b; Gatellier et al., 2000; Hur et al., 2004; Kim et al., 2004).

Chitosan is a cationic polysaccharide made from alkaline N-deacetylation of chitin. The component has multifunctional properties. including antibacterial (Sudarshan et al., 1992; Xie et al., 2001; Jia et al., 2002) fungicidal (Allan and Hadwiger. 1979). and antioxidant functions (Xie et al., 2001; Jeon et al., 2003). On the other hand. early studies showed that alginate, the polymer of β-D-mannuronate and its C-epimer form of α-L-guluronate inhibited the production of NO and H_2O_2 (Mo et al., 2003), and consequently exhibited antioxidant and antitumor effects by improving metabolic activity (Michio and Terukazu. 1992).

Given the previous studies. it was reasonable to assume that if chitosan and/or alginate are deposited in muscle tissue. lipid oxidation and microorganism growth could be significantly retarded. Chun et al. (2003) demonstrated a possible use of chitosan-alginate complex in the pig industry by showing that more than 90% of piglets with diarrhea benefited from oral feeding of the complex. By applying an isotope tracing technique, we previously showed that approximately 0.43% of isotope-conjugated chitosan-alginate-Fe(II) complex (CAFC) was deposited in pig muscle tissue (Korean Department of Agriculture and Forestry. 2002). However. its effect on meat quality has not yet been evaluated. The objective of this study was to investigate the effects of dietary CAFC supplementation on carcass and meat qualities of pig *m. longissimus* during chiller ageing.

MATERIALS AND METHODS

Animals, experimental design and treatment

A total of 122 LYD (Landrace×Yorkshire×Duroc) pigs were sampled from an industrial population. Seventy-four pigs (32 gilts and 42 barrows) were administered to a 3 ml

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of dietary supplementation of CAFC, diluted in water, per day from 25 to 70 days of age, while the remaining 48 pigs (20 gilts and 28 barrows) were fed with the same commercial diet without the supplementation. After the experimental period, an ordinary feeding regime was applied for all pigs until approximately 110 kg of slaughter weight was reached. All pigs were slaughtered after being stunned by an electronic stunner (230 volts for 2.5 s) at an industrial abattoir, and placed at a 1°C chiller until the following day. CAFC was manufactured by polymerizing two volumes of chitosan (MW 2,000-3,000, Tahoon Chemicals, Korea) and one volume of alginate (MW 1,500-2,000, EcoBio Inc, Korea) with 3% of Fe_2SO_4 at 90°C for 2 h (Korean Department of Agriculture and Forestry, 2002).

Sampling and measurement of objective meat quality

The day following slaughter, carcass grade was evaluated by carcass graders from the Korean Animal Products Grading Service (APGS, 2001). To evaluate the effect of CAFC supplementation on stability in objective meat quality during chiller ageing, 20 barrows (10 of each treatment) were randomly sampled, and longissimus muscles (from the 7^{th} thoracic vertebrae to the last lumbar vertebrae) were taken from the left sides. The muscle samples were cut into 6 portions of ca. 150 g, vaccum packed, and randomly assigned to six ageing treatments (3, 7, 12, 16, 20 and 25 days post-mortem).

The pH was measured using a portable needle-tipped combination electrode (NWKbinar pH-K21, Germany) in the geometric center of the aged muscle. Objective meat color was determined using a Minolta Chromameter (CR300, Minolta, Japan) on a freshly cut surface after a 30-min blooming at 1°C. Water-holding capacity (WHC) was determined by a centrifugation method (Kristensen and Purslow, 2001), with the following modification. One (1) g of homogenized tissue was placed in a 2 ml centricon tube (VIDAS, France). The sample containing tube was than placed in a 50 ml centrifugation tube, heated in a 70°C water bath for 30 min, and centrifuged at 100 g (Hitatchi, SCR20BA, Japan) for 10 min at room temperature (ca. 18°C). WHC was expressed as a percentage of weight loss of sample tissue during the centrifugation. Percentage of water content was determined by weight loss of 5 g muscle tissue at 102°C for 24 h. Cooking loss was determined by calculating percentage of weight loss of 150 g meat sample during 40 min cooking at a 80°C water bath.

Fatty acid composition of longissimus muscle was determined according to the method described by Morrison and Smith (1964). Briefly, an aliquot of total lipid extract was methylated and fatty acid methyl esters were analyzed by a gas chromatograph (Varian 3,600), fitted with a fused silica capillary column (30 m×0.32 mm I·D, 0.25 μm film thickness). The injection port was at 250°C, and the detector was at 300°C. Results were expressed as percentages based on the total peak area. Thiobarbituric acid reactive substances (TBARS) was determined as described by Witte et al. (1970) and expressed as mg malonaldehyde equivalents/kg sample, using 1,1,3,3-tetramethoxypropane as a standard precursor of malonaldehyde. Percentage of TBARS inhibition was determined by applying the equation: Inhibition=[(1-TBARS value for the treated sample/TBARS value for the control sample)]×100. Volatile basic nitrogen (VBN) was determined as descried by Park et al. (1998). The effect of the dietary supplementation on objective meat quality within each ageing time was assessed by a T-test procedure at 5% probability level (SAS, 1997).

RESULTS AND DISCUSSION

Numerous studies have demonstrated that chitosan has antibacterial and fungicidal properties (Allan and Hadwiger, 1979; Jia et al., 2002), and that it reduces diarrhea in pigs (Chun, et al., 2003). Previous studies indicated that both alginate and chitosan brought about alteration in the osmotic pressure of microorganisms by binding phospholipid and anions on the cell membrane (Cohen et al., 1991; Tasi and Su, 1999). On the other hand, the effect of CAFC supplementation on pork quality during ageing has not been reported.

Table 1. The effect of dietary supplementation of chitosan-alginate-Fe(II) complex (CAFC) on carcass characteristics and carcass grade

		Gilt		Barrow	
		Control	CAFC	Control	CAFC
Number of pigs		20	32	28	42
Feeding period (day)		174.4±1.80	164.8±2.10	178.3±2.80	168.2±2.20
Carcass weight (kg)[1]		80.80±1.88	81.19±1.02	81.79±1.50	82.36±1.09
Backfat thickness (mm)[1]		16.15±0.94	17.38±0.76	19.96±0.78	20.93±0.62
Frequency of carcass grade (%)[2]	A	20.0	56.5	35.7	42.9
	B	55.0	21.9	50.0	50.0
	C	15.0	21.9	10.7	7.0
	D	10.0	0	3.6	0

[1] There was no significant effect of the treatment within each sex group ($p>0.05$).
[2] A: Highest grade, D: Lowest grade.

Table 2. The effects of dietary supplementation of chitosan-alginate-Fe(II) complex (CAFC) on changes in pH, objective meat color, water-holding capacity (WHC) and cooking loss (Cook loss) in *m. longissimus dorsi* during chiller ageing

		Day of ageing [1]					
		3	7	12	16	20	25
pH	Control	5.6±0.06	5.6±0.00	5.7±0.00	5.6±0.03	5.7±0.00	5.7±0.03
	CAFC	5.5±0.03	5.7±0.03	5.8±0.07	5.6±0.07	5.6±0.03	5.6±0.03
CIE L*	Control	54.4±0.32	56.5±2.21	57.4±2.10	56.8±1.7	56.8±0.64	57.5±0.82
	CAFC	54.6±0.85	53.1±2.43	55.6±1.81	57.4±1.6	57.7±1.06	55.2±0.63
CIE a*	Control	7.5±0.22	7.9±0.53	7.4±0.18	7.8±0.16	7.9±0.30	8.9±0.68
	CAFC	7.4±0.87	8.4±0.67	8.0±0.76	8.4±0.72	8.5±0.64	9.7±0.66
WHC (%)	Control	54.5±1.23	53.4±1.24	56.3±1.35	58.9±0.67	53.4±0.53	51.8±1.81
	CAFC	52.9±1.26	55.9±1.34	55.6±1.36	59.8±0.56	53.6±0.93	54.9±0.35
Cook loss (%)	Control	32.5±1.05	33.7±1.14	33.7±0.86	33.3±0.54	32.0±1.14	32.7±1.68
	CAFC	32.9±0.77	32.7±1.15	33.3±1.12	32.0±0.31	32.7±1.09	32.1±1.29

[1] There was no significant effect of the feed supplementation on all meat quality traits within each ageing time ($p>0.05$).

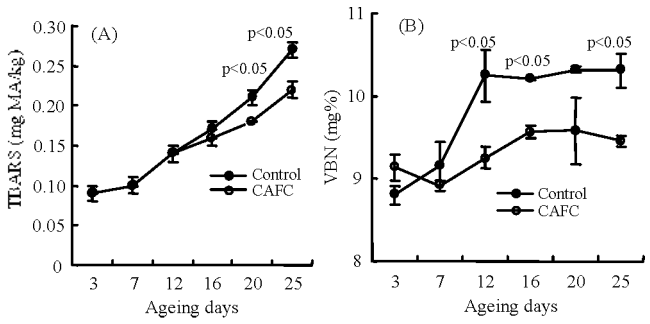

Figure 1. Effects of dietary supplementation of chitosan-alginate-Fe(II) complex (CAFC) on changes in (A) thiobarbituric acid reactive substances (TBARS) and (B) volatile basic nitrogen (VBN) in *m. longissimus dorsi* during chiller ageing. Bar: standard deviation.

Results in Table 1 show that CAFC-fed pigs required approximately 10 fewer feeding days than the control group, and that the treatment increases in the frequency of the grade A carcasses by approximately 35 and 7% for gilts and barrows, respectively. In the Korean pork grading scheme (APGS, 2001) pigs having an intermediate carcass weight with an intermediate backfat thickness receive a higher carcass grade. The results indicated that CAFC supplementation prevented both excessive backfat accumulation and extremely high carcass weight. This was also shown by the smaller standard deviations of carcass weight and backfat thickness for the treated group than those for the control group. In terms of the treatment effect on the carcass grade, the effect was considerably greater for gilt than barrow and that was of particular interest, but the mechanisms responsible were not clearly understood (Table 1).

To examine the treatment effect on stability of meat quality during chiller ageing, 20 barrows (10 pigs from each treatment), were randomly sampled. As seen in Table 2, the treatment had no significant effect on pH, meat color and WHC during ageing. pH at 24 h post-mortem has a direct effect on meat color and WHC through its effects on protein denaturation and the surface reflectance of muscle fiber (van Laack et al., 2001; Rosenvold and Andersen, 2003; Bertram et al., 2004; Hwang et al., 2004a). The studies implied that the objective meat qualities of meat color and WHC were largely related to post-mortem glycolytic rate (Hwang et al., 2004b). Given the fact, the current result of the similar pHs and objective meat qualities between the treatments indicated that the dietary supplementation did not influence post-mortem glycolytic rate and likely amount of energy resources at the time of slaughter.

TBARS values as indicators of lipid oxidation have been used by numerous research groups (*e.g.*, Kamil et al., 2002; Jeon et al., 2003). The most significant result of the current study was that the dietary supplementation significantly retarded lipid oxidation, as assessed by TBARS. As seen in Figure 1, meat from CAFC-fed pigs had significantly ($p<0.05$) lower TBARS values from 20 days of storage at 1°C, but at the practical storage temperature of 4°C, the beneficial effect would be detectable before that ageing time. Previous studies have shown that chitosan displays a significant antioxidative effect by chelating ferrous ions and consequently eliminating their prooxidant activity (Peng et al., 1998), and by combining with lipids (Xue et al., 1998). There is limited information about the biological effects of alginate. However, Mo et al. (2003) reported that alginate inhibited the production of NO and H_2O_2 by modulating production of the reactive oxygen/nitrogen components. The current study did not determine absorption and deposition rate of CAFC in *longissimus* muscle. However, we previously traced isotope-conjugated CAFC for pig, and found that a large portion of the feed additive was accumulated in the digestive organs, but also 0.43% of the fed-dose was detected in muscle tissue (Korean Department of Agriculture and Forestry, 2002). Given the apparent effect of both chitosan and alginate on antioxidant activity (Peng

Table 3. The effect of dietary supplementation of chitosan-alginate-Fe(II) complex (CAFC) on changes in fatty-acid compositions in *m. longissimus dorsi* during chiller ageing

	Day of ageing							
	3		12		20		25	
	Control	CAFC	Control	CAFC	Control	CAFC	Control	CAFC
C16:0	26.26±0.70	25.88±0.28	26.97±0.78	25.67±0.27	25.08±0.90	25.41±0.52	26.50^{a}±0.50	24.50^{b}±0.31
C18:0	13.01±0.81	12.54±0.10	12.46±0.55	11.75±0.21	11.49±0.29	12.07±0.13	13.41^{a}±0.20	12.24^{b}±0.37
C18:1n9	43.98±1.52	45.96±0.09	45.45±0.49	44.68±0.42	43.57±0.58	44.24±0.29	41.63^{b}±1.13	47.14^{a}±1.48
C18:2n6	10.51±1.80	9.33±0.19	9.31±1.68	12.09±0.56	14.92^{a}±0.70	12.82^{b}±0.46	13.43±1.24	11.14±0.78
C20:1n9	0.67±0.05	0.62±0.03	0.72^{a}±0.05	0.58^{b}±0.01	0.59±0.03	0.62±0.04	0.74^{a}±0.04	0.59^{b}±0.01
C20:2n6	0.33±0.05	0.26±0.01	0.25±0.03	0.30±0.02	0.36±0.02	0.34±0.03	0.40^{a}±0.03	0.28^{b}±0.01
C20:3n6	0.08±0.00	0.20±0.10	0.06^{b}±0.01	0.09^{a}±0.00	0.06^{b}±0.01	0.10^{a}±0.01	0.08±0.00	0.09±0.00
C20:4n6	0.31±0.02	0.32±0.12	0.30±0.02	0.32±0.01	0.27^{b}±0.03	0.36^{a}±0.01	0.20^{b}±0.01	0.35^{a}±0.03
∑SFA	40.78±1.05	39.99±0.23	41.08±1.34	39.02±0.16	37.95±1.18	39.07±0.44	41.42^{a}±0.45	37.99^{b}±0.57
∑PUFA	11.73±1.94	10.50±0.22	10.30±1.78	13.33±0.62	16.18^{a}±0.68	14.22^{b}±0.53	14.69±1.32	12.33±0.79
∑MUFA	47.49±2.10	49.52±0.22	48.62±0.82	47.65±0.53	45.87±0.51	46.72±0.36	43.89^{b}±1.25	49.68^{a}±1.36

[a, b] Means with different superscripts within the same ageing day of the same row differ significantly ($p<0.05$).

et al., 1998; Xue et al., 1998), it can be assumable that the current results suggest that the deposited CAFC maintained their functions to some extent during chiller ageing.

The rationale was further confirmed by fatty acid analysis (Table 3) as the sum of unsaturated fatty acids for the treatment group was significantly ($p<0.05$) higher than that for control group at 25 days of storage. Pork is susceptible to oxidation due to its high concentrations of polyunsaturated fatty acids compared to beef and lamb meats (Pearson et al., 1977). The unsaturated fatty acids undergo autoxidation by a free radical mechanism involving the abstraction of a labile hydrogen from the lipid molecule followed by the addition of molecular oxygen to the resultant lipid radical (Smith, 1981). In this experiment, the supplement resulted in significantly lower levels of C16:0, C18:0, C20:1n-9, C20:2n-6 than the control after 25 days at 1°C. On the other hand, the treatment showed significantly higher contents of C18:1n-9 and C20:4n-6 than the control, and consequently the sum of unsaturated fatty acids was significantly ($p<0.05$) higher in the CAFC-fed pigs after 20-d (Table 3) storage time. Given the well known function of chitosan and alginate, the result led us to postulate that the treatment reduced the formation of free radicals in fatty acids and the subsequent oxidation.

Another noticeable result was the significant effect of CAFC supplementation on VBN formation during ageing (Figure 1). VBN is a measurement of the nitrogen component of protein degradation, but also includes metabolite products such as AMP (Takasaka, 1975). Significantly lower levels of VBN after 12 days of storage for the CAFC-fed pigs suggests a significant reduction in protein degradation for that group. It has been well documented that structural and cytoplasmic proteins of meats are exposed to proteolytic actions of endogenous proteolysis during ageing, leading to the production of polypeptides (Hwang et al., 2003; Hwang et al., 2004c). The degradation of products consequently generate small peptides and free amino acids by subsequent actions of peptidases and aminopeptidases, respectively (Toldra and Flores, 2000). Furthermore activities of endo- and exo-proteases, -peptidases and -aminopeptidases are significantly affected by pH and temperature interactions during the onset of rigor mortis (Toldra and Flores, 2000; Hwang et al., 2004d). On the other hand, taken that negligible effect of the treatment on pH and temperature, significantly retarded formation of VBN for the CAFC-supplied pigs was unlikely related to the previously proposed mechanisms, and suggested that other beneficial characteristic was involved.

The current study did not determine the initial bacterial loads and their changes during ageing time. However, previous studies have demonstrated that chitosan and alginate had antibacterial functions (Michio and Terukazu, 1992; Jia et al., 2002), and that TBARS and VBN values were significantly increased when meat were contaminated by microorganisms (Branen, 1978; Park et al., 1998; Chae et al., 2004). It is possible that antibacterial activity of the supplement alone could have caused reduction in the formation of TBARS and VBN.

This then raises the question of whether reduced formation of TBARS (*i.e.*, lipid oxidation) was a consequence of direct effect of CAFC supplementation on antioxident activity in meat muscle. If antioxidant activity of CAFC was maintained in the tissue during ageing, it was reasonable to assume that the treatment had an effect on meat color changes during ageing. The status of myoglobin in meat is a significant determinant of color and autooxidation of oxymyoglobin (*e.g.*, in the Fe^{2+} oxidative state) results in the formation of a superoxide anion, leading to dark meat (Lee et al., 2003). However, there was no difference in objective meat color between the two groups, with a tendency of higher values for the CAFC-fed group.

In this regard, although the current study showed that dietary supplementation with CAFC effectively retarded the formation of TBARS and VBN, the biological mechanisms involved are not clear.

IMPLICATIONS

The current study showed that dietary supplementation of CAFC reduced the time for pigs to reach 110 kg by approximately 10 days, and resulted in higher carcass grades. In addition, the treatment effectively slowed down the formations of TBARS and VBN during chiller ageing. However, whether these were direct effects of antioxidant properties of chitosan and/or alginate, or a consequence of their antibacterial functions is unknown

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