

## **Chapter 8**

### **Final Discussion and Conclusions**

The human gut microbiota has a huge metabolic activity which can be considered as an additional organ (Bocci, 1992; O'Hara & Shanahan, 2006). This collective metabolic activity includes the degradation of dietary constituents for which the host lacks digestive capacity and the production of nutrients and vitamins (Conly *et al.*, 1994; Hill, 1997; Miyazawa *et al.*, 1996; Roberfroid *et al.*, 1995). Furthermore, it has been demonstrated that a number of the metabolites produced by the commensal enteric microbiota possess functional properties for the host. Conjugated linoleic acid isomers (CLA) are microbial metabolites which have shown potential in the treatment of some of the most prevalent diseases in the Western world including cancer, diabetes, obesity, and cardiovascular disease (Belury, 1999; Bhattacharya *et al.*, 2006; Miller *et al.*, 2002; Wahle *et al.*, 2004). It has been shown that selected strains of commensal lactobacilli and bifidobacteria can bioconvert linoleic acid to CLA both *in vitro*, *ex-vivo* and *in vivo* (Hennessy *et al.*, 2007; Sieber *et al.*, 2004; Wall *et al.*, 2009).

Under normal experimental conditions CLA production by Bifidobacteria is characterised in synthetic medium (Coakley *et al.*, 2003; Jiang *et al.*, 1998). However, given the expense the large production of CLA in these synthetic medium is not a viable option (Ventling & Mistry, 1993). A potentially more useful medium would be milk, however, given the difficulty of propagating bifidobacteria in milk and the relationship between cell numbers and CLA production, the effective and efficient production of CLA in milk by bifidobacteria would appear difficult (Kim *et al.*, 2000; Poch & Bezkorovainy, 1988).

Results from Chapter 2 demonstrated the potential of reconstituted skimmed milk (RSM) as a medium for microbial production of CLA from free linoleic acid by bifidobacteria. In the past, fermented milks and yoghurts have proven themselves effective and efficient mediums both for the delivery of probiotic

bacteria to the human gastro-intestinal tract and as media for the production of CLA by strains of propionibacteria and lactobacilli (de Vrese & Schrezenmeir, 2008; Parvez *et al.*, 2006; Xu *et al.*, 2005). We observed that RSM alone did not support growth and CLA production by strains of *B. breve* and *B. longum*, but that via supplementation of this medium with yeast extract, sodium acetate and the prebiotic Raftiline GR (Inulin) microbial CLA production by strains of bifidobacteria was significantly increased to concentrations comparable to those achieved in the synthetic medium de Man, Rogosa and Sharpe supplemented with L-cysteine hydrochloride (cys-MRS). Benefits associated with using a probiotic capable of converting linoleic acid to CLA in a fermented milk have been demonstrated. When enriched in a dairy food during fermentation using active cultures, the CLA produced would then be readily available for absorption in the intestine, thus benefiting the prognosis of conditions such as cardiovascular disease, cancers, obesity and diabetes (Iqbal & Hussain, 2009; Tsuzuki & Ikeda, 2007). Furthermore, introduction of CLA producing bifidobacteria to the large intestine offers the potential for the direct and continuous provision of CLA *in situ* (Wall *et al.*, 2009). Indeed, reports such as that of Edionwe & Kies (2001) have indicated that as much as 20 mg of linoleic acid may be excreted by man on a daily basis, indicating that there is an abundant supply of substrate to facilitate *in vivo* CLA production. This CLA when produced in the colon has the potential to be advantageous in the treatment of colonic conditions such as inflammatory bowel disease and colon cancer through anti-inflammatory and anti-proliferative activities (Bassaganya-Riera *et al.*, 2002; Bassaganya-Riera *et al.*, 2004; Bhattacharya *et al.*, 2006; Wahle *et al.*, 2004).

The development of a milk based medium capable of sustaining the growth and CLA production by bifidobacteria in milk paves the way for further

investigations into the potential of natural CLA enriched bifidus milks to improve human health. Furthermore, this CLA enriched RSM might also have applications as a functional ingredient which could be directly added to sprayed dried powders or added to cheeses and yoghurts.

In Chapter 3 we reported the potential of strains of CLA producing bifidobacteria and propionibacteria to bioconvert a range of unsaturated fatty acids to their respective conjugated isomers. Previous studies have demonstrated the potent bioactive properties of various natural conjugated fatty acids including the CLA isomers, and conjugated  $\alpha$ -linolenic acid (CALA) and synthetic conjugated isomers conjugated eicosapentaenoic acid (EPA) and conjugated docosahexaenoic acid (DHA) (Bhattacharya *et al.*, 2006; Coakley *et al.*, 2009; Danbara *et al.*, 2004; Tsuzuki *et al.*, 2004b; Tsuzuki *et al.*, 2007). Thus, the discovery and identification of novel conjugated fatty acids would appear to be of significant interest.

In our second study we demonstrated the ability of strains of *Propionibacterium* and *Bifidobacterium* to conjugate  $\alpha$ -linolenic acid to CALA,  $\gamma$ -linolenic acid to conjugated  $\gamma$ -linolenic acid (CGLA) and stearidonic acid to conjugated stearidonic acid (CSA). These conjugated fatty acids were isolated from the other fatty acids present in the fermentation medium by RP-HPLC and identified by GLC-MS. Of the strains assayed *B. breve* DPC6330 was identified as being particularly effective in the production of these conjugated isomers. This strain produced the conjugated fatty acids primarily during the logarithmic phase of cellular growth and displayed a preference for conjugating fatty acid substrates in the order  $\alpha$ -linolenic acid > linoleic acid >  $\gamma$ -linolenic acid > stearidonic acid. Like the other conjugated fatty acids currently under investigation it is highly likely that CALA, CGLA and CSA may also possess potent bio-activity. The ability of dairy

*Propionibacterium* and intestinally isolated *Bifidobacterium* to produce CALA, CGLA and CSA offer the prospect of the enrichment of milk and yoghurt with these fatty acids through fermentation as outlined in Chapter 1 for CLA, or the establishment of microbiota in the human gastro-intestinal tract capable of producing these fatty acids *in vivo*. Indeed, there is already *in vivo* evidence from studies to indicate that CALA isomers are absorbed in the intestine (Plourde *et al.*, 2006; Tsuzuki *et al.*, 2003; Tsuzuki *et al.*, 2004c).

Investigations into the bioactivity of conjugated fatty acids such as CLA, CALA, conjugated EPA and conjugated DHA have demonstrated that these fatty acids have vast potential in the treatment of a number of diseases prevalent in the Western world. Indeed there is both *in vivo* and *in vitro* evidence associating these fatty acids with anti-carcinogenic, anti-atherosclerotic, anti-diabetogenic, and anti-adipogenic activities which might be shared by the microbially produced CALA, CGLA and CSA isomers. Given this vast potential further studies into the impact of these isomers on such conditions both *in vitro* and *in vivo* are required.

The anti-carcinogenic properties of the conjugated unsaturated fatty acids CALA, CGLA and CSA were investigated in Chapter 4 of this thesis. We demonstrated that CALA, CGLA and CSA exerted an anti-proliferative activity against the SW480 colon cancer cell line which was significantly greater than that displayed against the normal fetal human colonic epithelial FHC cell line. Despite its anti-proliferative properties against the SW480 cell line the study demonstrated that the activity of CALA was not significantly greater than that of  $\alpha$ -linolenic acid. Unlike CALA the anti-proliferative activity of both CGLA and CSA against the SW480 cell line was significantly greater than that of their respective parent unsaturated fatty acids,  $\gamma$ -linolenic acid and stearidonic acid. Exposure of the

SW480 cell line to CALA resulted in reductions in the ratio of  $\omega$ -6 to  $\omega$ -3 polyunsaturated fatty acids (PUFA) found in the cellular phospholipids, which could reduce the inflammatory status of the cell, and to reductions in the cellular concentration of the anti-apoptotic oncoprotein Bcl-2. Reductions in the inflammatory status of the cell are generally associated with reduced cancer cell proliferation, while reductions in the concentration of cellular Bcl-2 are associated with increased cellular apoptosis, indicating that CALA may mediate its anti-carcinogenic activity through a multiple mechanisms. CGLA displayed a number of cytotoxic properties towards the SW480 and FHC cell lines and its anti-carcinogenic activity was found to be strongly related to increased lipid peroxidation similarly to other conjugated fatty acids (Tsuzuki *et al.*, 2004a; Tsuzuki *et al.*, 2007) (Chapter 1.3). Although the isomer was not incorporated into the cellular phospholipids, exposure of the SW480 cells to the conjugated fatty acid significantly reduced the ratio of  $\omega$ -6 to  $\omega$ -3 PUFA in the cell membrane, potentially reducing the inflammatory status of the cell. Like CGLA, CSA also displayed cytotoxic properties against both the SW480 and FHC cell lines which were strongly associated with increased cellular lipid peroxidation, although reduced cellular concentrations of the anti-apoptotic oncoprotein Bcl-2 may also have played a significant role. Exposure of the SW480 cell line to CSA resulted in a significant decrease in the ratio of  $\omega$ -6 to  $\omega$ -3 PUFA in the cell membrane primarily as a result of the uptake of the conjugated fatty acid by the cell. Increased cellular lipid peroxidation has been extensively linked with the anti-carcinogenic activity of conjugated fatty acids, as seen in the present study (Suzuki *et al.*, 2001; Tsuzuki *et al.*, 2004a; Tsuzuki *et al.*, 2007). Similarly to increased cellular lipid peroxidation, the ability of conjugated fatty acids to modulate the expression of pro and anti-apoptotic oncogenes has been linked with their anti-carcinogenic properties.

Reduced expression of anti-apoptotic oncogene Bcl-2 and its oncoprotein have been the most extensively reported, corresponding well with our observations in this study (Beppu *et al.*, 2006; Yasui *et al.*, 2005; Yasui *et al.*, 2006).

The production of conjugated fatty acids with inhibitory activity against colonic cancers by a microbe normally abundant in the colon presents an opportunity for the *in situ* production of a bioactive at its target site. As the substrate fatty acids from which CALA, CGLA and CSA (i.e.  $\alpha$ -linolenic acid,  $\gamma$ -linolenic acid and stearidonic acid, respectively) are commonly found in plants that constitute part of the human diet, hence, it is likely that their *in vivo* production occurs within the gastro-intestinal tract (Fan & Chapkin, 1998; Li *et al.*, 2003; Whelan, 2009). Further credence is given to this theory in light of the recent evidence pertaining to the *ex vivo* and *in vivo* production of CLA from dietary linoleic acid (Ewaschuk *et al.*, 2006; Wall *et al.*, 2009). In addition, the use of such bioactive microbes in fermented probiotic dairy products offers a potential vehicle for their delivery to the gastro-intestinal tract (Sieber *et al.*, 2004; Xu *et al.*, 2004; Xu *et al.*, 2005).

While these preliminary investigations have highlighted the potential of these conjugated fatty acids in the treatment of colon cancer, further *in vitro* and *in vivo* work is required to determine the overall efficacy of these fatty acids in the treatment of colonic cancers and other tumors. Furthermore, work is required to determine what other functional properties these conjugated fatty acids possess with regard to the treatment of conditions such as atherosclerosis, obesity and diabetes.

Conjugated fatty acids have also been shown to exhibit a range of bioactive properties including anti-carcinogenic, anti-atherosclerotic, anti-diabetogenic and anti-obesogenic activity,, while more recent investigations have also shown that the

CLA isomers possess potent anti-microbial activity against *Staphylococcus aureus* (Belury, 2002; Bhattacharya *et al.*, 2006; Kelsey *et al.*, 2006; Wahle *et al.*, 2004). Indeed, it has been shown that both CLA and its unsaturated parent fatty acid, linoleic acid, exhibit anti-microbial activity against *Staphylococcus aureus* (Greenway & Dyke, 1979; Kelsey *et al.*, 2006). In Chapter 5 of this thesis, the anti-microbial activity of three novel fatty acids, CALA, CGLA and CSA, and their respective parent unsaturated fatty acids,  $\alpha$ -linolenic acid,  $\gamma$ -linolenic acid, and stearidonic acid, against the methicillin resistant *S. aureus* (MRSA) strain ATCC 43300 was investigated. While the conjugated fatty acids displayed strong inhibitory activity against MRSA, the profile of inhibitory activity differed substantially from that of their parent unsaturated fatty acids. The development of resistance to antibiotics is a major obstacle to their effectiveness in the treatment of infection (EARSS, 2006). We observed that neither exposure to the conjugates nor their parent unsaturated fatty acids encouraged the development of resistance in the strain of MRSA assayed. Though the mechanism behind the anti-microbial activity of the conjugated fatty acids or their parent unsaturated fatty acids was not elucidated in the current study, previous investigations have associated this anti-microbial activity with increases in the permeability of the cell membrane, increases in cellular lipid peroxidation, and/or the disruption of cell energetics (Butcher *et al.*, 1976; Kenny *et al.*, 2009; Knapp & Melly, 1986; Raychowdhury *et al.*, 1985). Although potent *in vitro* inhibitors of *S. aureus*, concern as to efficacy of fatty acids to inhibit *S. aureus in vivo* have arisen due to their reported loss of activity in the presence of blood serum (Lacey & Lord, 1981). In this study we observed that the parent unsaturated fatty acid and their conjugated isomers both remained active in the presence of fetal bovine serum (1% v/v). However, it was observed that FBS had a stimulatory effect on the growth of *S. aureus in vitro*.



Overall, the observations of this study suggest that conjugated fatty acids and their parent unsaturated fatty acids can be successful in the *in vitro* treatment of MRSA and have the potential to be effective *in vivo*. However, further work is required in detailing the mechanisms through which this anti-microbial activity is mediated and if the fatty acids are effective in the treatment of more than one strain of MRSA. Leading from these studies there is the potential for investigations into the *in vivo* activity of these fatty acids in animal models.

The objective of Chapter 6 was to determine the impact of dietary supplementation with  $\omega$ -6 and  $\omega$ -3 PUFA on the concentration of CLA found in the plasma of Holstein Friesian heifers. Recent studies have suggested that dietary supplementation of Holstein Friesian cows with CLA isomers may be capable of offsetting the high proportion of embryonic loss which occurs in cattle during the first three weeks of pregnancy (Castaneda-Gutierrez *et al.*, 2007; Rodriguez-Sallaberry *et al.*, 2006). The  $\omega$ -6 PUFA supplemented diet (linoleic acid rich) increased the concentrations of linoleic acid in plasma, consistent with previous studies (Burns *et al.*, 2003; Filley *et al.*, 2000). In our study, the increased supply of linoleic acid did not significantly increase the concentration of *c*9, *t*11 CLA isomer, *t*10, *c*12 CLA isomer or the CLA precursor vaccenic acid found in the blood plasma. This observation is contrary previous studies in cows where the increased supply of dietary linoleic acid was associated with increased concentrations of *c*9, *t*11 CLA isomer and vaccenic acid in plasma and milkfat of ruminants (Dhiman *et al.*, 2000; Dhiman *et al.*, 2005; Loor & Herbein, 2003). The  $\omega$ -3 PUFA enriched diet (fish oil) was found to increase plasma concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in agreement with previous studies involving dietary enrichment with either fish oil (Mattos *et al.*, 2004) or fish meal

(Burns *et al.*, 2003; Wamsley *et al.*, 2005). The inclusion of  $\omega$ -3 PUFA in the diet in the form of fish oil did not significantly increase the concentration of the *c*9, *t*11 CLA isomer found in the plasma. However, the concentration of *t*10, *c*12 CLA in the plasma was significantly higher in the  $\omega$ -3 PUFA fed group compared with the control and the  $\omega$ -6 PUFA fed groups. Despite an overall increase in plasma CLA concentrations as a result of the provision of the  $\omega$ -3 PUFA enriched diet, increases of the magnitude of those observed in cows on similar diets in previous studies were not observed (Loor *et al.*, 2005). The lower concentrations of CLA found in the present study may be a result of the relative immaturity of the mammary gland in the heifers used in , one of the major sources of endogenous CLA production, of the heifer relative to the cow (Lal & Narayanan, 1984; Stanton *et al.*, 1997).

In this study, we found significant increases in the concentration of the health promoting fatty acids EPA and DHA in the plasma when animals were fed a diet supplemented with fish oil. Like CLA, the provision of these fatty acids in the bovine diet has also been demonstrated to improve fertility (Mattos *et al.*, 2002; Mattos *et al.*, 2004). In the second part of this study, we investigated the impact of the provision of a ruminally protected  $\omega$ -3 PUFA rich fish oil supplement on the fatty acid profile of plasma, endometrial tissue and follicular fluid of Holstein Friesian heifers when provided at varying concentrations. The plasma EPA concentrations of the  $\omega$ -3 PUFA supplemented animals increased in a dose dependent manner as did the EPA concentrations of endometrial tissue, which is consistent with previous studies (Burns *et al.*, 2003; Wamsley *et al.*, 2005). Spain *et al.* (1995) previously reported linear increases in plasma EPA in dairy cows fed increasing concentrations of fish meal. Increases in the concentration of DHA in both plasma and endometrial tissue found in the present study are consistent with some previous reports (Burns *et al.*, 2003; Wamsley *et al.*, 2005). Although there

was a positive relationship between dietary and plasma concentrations of this  $\omega$ -3 PUFA and the concentration found in the uterine endometrium, the relationship was not as strong as that of EPA. Similarly, other studies have found that increasing tissue DHA concentrations is not readily achievable through dietary manipulation (Burns *et al.*, 2003; Wamsley *et al.*, 2005). It is important to emphasise that while endometrial DHA concentrations in the current study were similar to those reported for uterine caruncular (Mattos *et al.*, 2004) and endometrial tissue (Moussavi *et al.*, 2007), the EPA and total  $\omega$ -3 PUFA concentrations reported here are several multiples higher than the concentrations reported in the aforementioned studies. Therefore it would appear that the provision of a ruminally protected fish oil in the diet of heifers is an excellent strategy for increasing the concentration of PUFA found in targets associated with the reproductive process. The linoleic acid concentration of both plasma and endometrial tissue was reduced in a linear fashion by increasing  $\omega$ -3 PUFA supplementation and is consistent with the observations of others (Burns *et al.*, 2003; Childs *et al.*, 2008; Mattos *et al.*, 2004). Plasma concentrations of arachidonic acid increased with increasing  $\omega$ -3 PUFA supplementation. This, again is similar to the report of Burns *et al.* (2003) and with more recent work from our laboratory (Childs *et al.*, 2008). However, despite the increase in plasma concentrations of arachidonic acid the endometrial concentrations of this fatty acid were reduced with increasing level of dietary  $\omega$ -3 PUFA supplementation. The higher plasma concentrations of arachidonic acid may be a function of either higher concentrations available in the diet, or due to the ability of both EPA and DHA to displace arachidonic acid in membrane phospholipids (Mattos *et al.*, 2003), resulting in a reduction in tissue uptake and a relative 'accumulation' of arachidonic acid in plasma. While the majority of fatty acids in follicular fluid were unaffected by the  $\omega$ -3 PUFA supplementation, EPA

concentrations in the fluid increased in a linear and quadratic fashion while linoleic and oleic acid concentrations both decreased linearly. Furthermore, a strong positive relationship was found between the concentrations of a number of  $\omega$ -3 and  $\omega$ -6 PUFA in follicular fluid and their concentrations in plasma which may impact on the inflammatory status of the animal.

The objective of chapter 7 was to determine if the inclusion of a ruminally protected  $\omega$ -3 PUFA in the diet of Holstein Friesian heifers could be utilised to improve the nutritional quality of the fatty acids found in the animal's meat. Recent studies have demonstrated the increasing importance of red meat in our daily PUFA intake thus tailoring the type of PUFA found in these tissues is likely to be of increasing importance (Howe *et al.*, 2006; Howe *et al.*, 2003). Indeed, given that red meat contains an abundance of fatty acids perceived as being negative to human health such as saturated fatty acids (SFA), *trans* fatty acids and  $\omega$ -6 PUFA, the incorporation of health promoting  $\omega$ -3 PUFA at the expense of these detrimental fatty acids would appear desirable (Cross *et al.*, 2008; Hu *et al.*, 1999; Simopoulos, 2002). In the current study, the provision of the  $\omega$ -3 PUFA supplement in the diet of Holstein Friesian heifers substantially improved the overall fatty acid profile of the plasma, adipose, liver, muscle and mammary tissues. These improvements were mediated through significant increases in the concentration of EPA and DHA, which constituted the major  $\omega$ -3 PUFA in the  $\omega$ -3 PUFA enriched diet. Reductions in the concentration of total SFA were observed in the plasma, liver, muscle and mammary tissues primarily as a result of reductions in the concentration of palmitic and stearic acids. The reductions in SFA concentrations may be a result of the increased competition provided by the higher total  $\omega$ -3 PUFA provided by the diet

or alternatively as a result of the potent suppressive effects of  $\omega$ -3 PUFA and in particular EPA and DHA on lipogenesis in tissues such as the liver (Jump *et al.*, 1994; Jump *et al.*, 1996; Jump *et al.*, 1999). The  $\omega$ -3 PUFA enriched diet also improved the ratio of PUFA to SFA, a major indicator of the fatty acid quality of red meat. Indeed, the ratio of PUFA to SFA was increased by 1.14 fold in the adipose tissue, and by 1.75 fold in the muscle tissue of animals receiving the  $\omega$ -3 PUFA enriched diet and correlate well with the observations of others (Mach *et al.*, 2006; Scollan *et al.*, 2001). Furthermore, the ratio of PUFA to SFA in the liver tissue was increased above the 0.45 advised by the British Department of Health (1994). The provision of the  $\omega$ -3 PUFA supplement in the heifers diet significantly reduced the concentration of total  $\omega$ -6 PUFA in the plasma and liver tissue of animals receiving the diet. Such reductions were attributed to the competition provided by  $\omega$ -3 PUFA with  $\omega$ -6 PUFA for incorporation into the membrane phospholipids of the cells, or alternatively to the interference of the  $\omega$ -3 PUFA with the endogenous synthesis of eicosatrienoic and arachidonic acids (Li *et al.*, 2003; Ratnayake *et al.*, 1989; Scollan *et al.*, 2001). The increases in the concentration of  $\omega$ -3 PUFA and reductions in the concentration of  $\omega$ -6 PUFA served to reduce the overall ratio of  $\omega$ -6 to  $\omega$ -3 PUFA found in the tissues. In humans reducing the ratio of  $\omega$ -6 to  $\omega$ -3 PUFA has been associated with reducing the pathogenesis of diseases such as cancer, cardiovascular diseases and diabetes, which have become associated with the increased prevalence of  $\omega$ -6 PUFA in the human diet (Astorg, 2004; Bagga *et al.*, 1997; Simopoulos, 2006; Simopoulos, 2008). In the current study the ratio  $\omega$ -6 to  $\omega$ -3 PUFA in the muscle and liver tissue were significantly lower than the ratio of 4:1 associated with a 70% reduction on total mortality from cardiovascular disease, the ratio of 2.5:1 associated with the reduced proliferation of colorectal

cancer, the ratio of 2-3:1 which suppressed inflammatory rheumatoid arthritis, and the ratio of 5:1 which had a beneficial impact on asthma sufferers, in animals on the  $\omega$ -3 PUFA enriched diet (Simopoulos, 2002).

Importantly strong relationships between the increases seen in EPA, DHA, and total  $\omega$ -3 PUFA concentrations in the plasma and those observed in the tissues were found. Further relationships were also found between the plasma and the liver tissue with regard to the ratio of PUFA to SFA and between the plasma and the muscle and liver tissue with regard to the ratio of  $\omega$ -6 to  $\omega$ -3 PUFA of animals receiving the  $\omega$ -3 PUFA enriched dietary supplement. As both the ratio of PUFA to SFA the ratio of  $\omega$ -6 to  $\omega$ -3 are important indicators of the overall fatty acid quality of meat, such a correlation in muscle and liver tissue may be extremely important, permitting the determination of the fatty acid quality of two of the most important bovine tissues with regard to human dietary consumption from a plasma sample.

In conclusion the main findings of this thesis have demonstrated the ability of CLA producing Bifidobacteria to produce CLA in a milk based medium at concentrations comparable to those achieved in synthetic medium. We have also demonstrated that these CLA producing bifidobacteria possess the capability to conjugate a range of other C18 unsaturated fatty acids, which possess potent anti-carcinogenic against the SW480 colon cancer cell line and anti-microbial activity against methicillin resistant *S. aureus in vitro*. Finally, we have shown how the provision of an  $\omega$ -3 PUFA enriched supplement in the diet of Holstein Friesian heifers can be utilised to improve the fatty acid composition of key targets associated with bovine reproduction potentially reducing the inflammatory status of the animal. It was also shown how this diet could also benefit the overall quality of bovine meat, enhancing the fatty acid quality of tissues such as the muscular tissue

and liver tissue and improving indices of meat quality such as the ratio of PUFA to saturated fatty acids and the ratio of  $\omega$ -6 to  $\omega$ -3 PUFA.

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