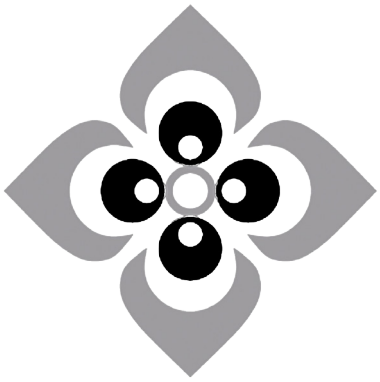

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Module on

Role of enzymes in food processing

By

Shugufta Rasheed

Research Scholar

Department of Botany

University of Kashmir - 190006

Enzymes

Enzymes are biological catalysts that promote most of the biochemical reactions which occur in the cell. As is typical for catalysts they speed up biochemical reactions without being consumed in the process. Depending on the type of reaction catalyzed, enzymes are divided into six main classes:

Oxidoreductases: Transfer of electrons from one substrate molecule to another (e.g., dehydrogenases, reductases, oxidases).

Transferases: Transfer of functional group from one substrate molecule to another (e.g., glycosyl transferases, acetyl transferases and aminotransferases).

Hydrolases: Transfer of functional group from substrate to water (e.g., glycoside hydrolases, peptidases, esterases).

Lyases: Elimination of functional group from substrate with the formation of double bonds. Thus, bonds are cleaved using a different principle than hydrolysis (e.g., pectate lyases break glycosidic linkages by beta-elimination).

Isomerases: Transfer of groups from one position to another in the same molecule (e.g., glucose isomerase).

Ligases: Addition of function group to substrate usually coupled with ATP hydrolysis (e.g., glycine – tRNA ligase).

Enzymes play key roles in numerous biotechnology products and processes that are commonly encountered in the form of food and beverages, cleaning supplies, clothing, paper products, transportation fuels, pharmaceuticals and monitoring devices. The presence of enzymes in food is very common with oxireductases and hydrolases being the most important ones. In the oxireductase group, the enzymes that are of interest for the food industry are phenolases, peroxidases, catalases, peroxidases and lipoxygenases. The use of enzymes in food processing is one of the greatest impacts of biotechnology on the sector. The importance of enzymes is likely to increase as consumers demand more natural products free of chemical additives. For example, enzymes can be used to replace potassium bromate, a chemical additive that has been banned in a number of countries. Enzymes extracted from edible plants and the tissues of food animals, as well as those produced by microorganisms (bacteria, yeasts, and fungi), have been used for centuries in food manufacturing. Rennet is an example of a natural enzyme mixture from the stomach of calves or other domestic animals that has been used in cheese making for centuries. Rennet contains a protease enzyme that coagulates milk, causing it to separate into solids (curds) and liquids (whey). Alternatively, for centuries enzymes produced by

yeast have been used to ferment grape juice in order to make wine. Today, enzymes are used in bakery, brewing, dairy, meat, sugar, fruit processing and other food industries. Various enzymes used are amylases, proteases, lipases, glucooxidases, pectinases and tannases and many others. Food spoilage, through enzyme produced by spoilage microorganisms, are also of concern as the shelf life of the food is affected. Controlling enzymatic activity is critical during food processing as enzymes have both beneficial and detrimental effects. Knowledge on enzyme stability under the relevant processing conditions is required for process design (e.g. its thermostability, resistance towards acid environment). In case of detrimental enzymatic action, elimination or retardation of the enzymatic reaction is performed by inactivation of the enzymes either by physical methods (e.g., heating, blanching) or by chemical additives. Enzyme manufacturers have optimized microorganisms for the production of enzymes through natural selection and classical breeding techniques. Direct genetic modification (biotechnology) encompasses the most precise methods for optimizing microorganisms for the production of enzymes. These methods are used to obtain high yielding production organisms. Biotechnology also provides the tools to have a genetic sequence from a plant, animal, or a microorganism, from which commercial

scale enzyme production is not adequate, to be transferred to a microorganism that has a safe history of enzyme production for food use. Enzymes produced through biotechnology are identical to those found in nature. Additionally, enzymes produced by microorganisms are extracted and purified before they are used in food manufacturing. The world wide application of these will increase by developing tailor made enzymes through recombinant DNA technology which could enhance the yields and modify their specificity to meet consumer's requirement in terms of cost, calorie and taste.

Enzymes in fruit juice processing

The demand of fruit juices is increasing day by day as a result of increasing health awareness among the people. Most of juice extraction processes are not producing satisfactory quantity and quality of juices. Enzymatic extraction of juices results in higher yield. Enzymes can play important roles in preparing and processing various fruit and vegetable juices such as apple, orange, grapefruit, cranberry, pineapple, grape, carrot, and lemon. Fruits and vegetables are particularly rich in pectic substances. Pectin, a hydrocolloid, has a great affinity for water and can form gels under certain conditions. The addition of exogenous enzymes, such as pectinases, pectin lyase, pectin esterase and polygalacturanase,

reduces viscosity and improves pressability as the pectin gel collapses. For complete liquefaction of fruits and vegetables, hemicellulases, cellulases and amylases can be used in addition to pectinases. Arabinan, a linear polymer of arabinofuranose units with 1,5 linkages, is an important component of fruit cell walls and may cause haze in fruit juice concentrate. Haze formation can be prevented by using endo-arabinanases to break down arabinan into soluble, low molecular weight degradation products (arabinofurano-oligosaccharides). Various commercial enzyme preparations are currently available for such use in the fruit juice industry. Enzymatic de-bittering of grapefruit juice can be achieved through the application of fungal naringinase preparations. The enzyme preparation contains both alpha-rhamnosidase and beta-glucosidase activities. Alpha-Rhamnosidase first breaks down naringin, an extremely bitter flavanoid, to rhamnose and prunin and then beta-glucosidase hydrolyzes prunin to glucose and naringenin. Prunin bitterness is less than one-third of that of naringin. However, alpha-rhamnosidase is competitively inhibited by rhamnose and beta-glucosidase is inhibited by glucose. Immobilized enzymes are used in flow-through reactors to solve the inhibition problems. Naringenin is reported to provide antioxidant and other beneficial effects to human health. Another

enzyme, the flavoprotein glucose oxidase, is used to scavenge oxygen in fruit juice and beverages to prevent color and taste changes upon storage. Glucose oxidase is produced by various fungi such as Aspergillus niger and Penicillium purpurogenum.

Enzymes in dairy processing

The proteolytic system of lactic acid bacteria is essential for their growth in milk and contributes significantly to flavour development in fermented milk products. The proteolytic system is composed of proteinases which initially cleaves the milk protein to peptides; peptidases which cleave the peptides to small peptides and amino acids and transport system responsible for cellular uptake of small peptides and amino acids. Lactic acid bacteria have a complex proteolytic system capable of converting milk casein to the free amino acids and peptides necessary for their growth. These proteinases include extracellular proteinases, endopeptidases, aminopeptidases, tripeptidases and proline specific peptidases, which are all serine proteases. Aminopeptidases are important for the development of flavour in fermented milk products, since they are capable of releasing single amino acid residues from oligopeptides formed by extracellular proteinase activity. Rennets (rennin, a mixture of chymosin and pepsin obtained mainly from animal and microbial sources) are used for coagulation of milk in

the first stage of cheese production.

Other enzymes used for dairy food application include: (i) proteases to reduce allergic properties of cow milk products for infants, and (ii) lipases for development of lipolytic flavours in speciality cheeses. The functional properties of milk proteins may be improved by limited proteolysis through the enzymatic modification of milk proteins. An acid soluble casein, free of off flavour and suitable for incorporation into beverages and other acid foods, has been prepared by limited proteolysis. The antigenicity of casein is destroyed by proteolysis, and the hydrolysate is suitable for use in milk protein based foods for infants allergic to cow milk.

Enzymes in baking processing

For decades, enzymes such as malt and fungal alpha amylases have been used in bread making.

Rapid advances in biotechnology have made a number of exciting new enzymes available for the baking industry. The dough for bread, rolls, buns and similar products consists of flour, water, yeast, salt and possibly other ingredients such as sugar and fat. Flour consists of gluten, starch, non starch polysaccharides, lipids and trace amounts of minerals. As soon as the dough is made, the yeast starts to work on the fermentable sugars, transforming them into alcohol and carbon dioxide, which makes the dough

rise. The main component of wheat flour is starch. Amylases can degrade starch and produce small dextrins for the yeast to act upon. There is also a special type of amylase that modifies starch during baking to give a significant antistaling effect. Gluten is a combination of proteins that forms a large network during dough formation. This network holds the gas in during dough proofing and baking. The strength of this gluten network is therefore extremely important for the quality of all bread raised using yeast. Enzymes such as hemicellulases, xylanases, lipases and oxidases can directly or indirectly improve the strength of the gluten network and so improve the quality of the finished bread.

Enzymes in the modification of fats and oils

Lipases are a ubiquitous class of enzymes which catalyze hydrolysis, esterification (synthesis), and transesterification (group exchange of esters). These enzymes are involved in fat hydrolysis, flavor development in dairy products, transesterification of fats and oils and production of chiral organic compounds. Lipolytic reactions occur at the lipid–water interface. There are two broad types of lipases based on their positional specificity. Nonspecific lipase releases fatty acids from all three positions of the glycerol moiety and is used to hydrolyze fats and oils completely to free fatty acids and glycerol. These are produced by Candida sp., Staphylococcus

sp., and Geotrichum sp. The other type of the enzyme is 1, 3-specific lipase which releases fatty acids from 1,3 positions and preferentially free fatty acids and di- and monoglycerides as the reaction products. This type of lipase is produced by Aspergillus, Mucor, Rhizopus and Pseudomonas sp.

Conventional fat hydrolysis by high-temperature steam splitting (250–260^{o}C at 50 bar) consumes large amounts of energy and creates environmental concerns. Lipases can be used to hydrolyze fats and oils with excellent yields, but the high cost of the enzymes, exacerbated by enzyme stability problems, currently makes the process uneconomical. Similarly, lipase-catalyzed transesterification of plant glycerides to make alkyl esters (plus glycerol byproduct) for use as biodiesel and replace base-catalyzed processes would be a huge market for enzyme biotechnology. In the meantime, higher value and lower bulk products such as edible and nonedible fats and oils with specialized, improved, or new properties are produced by the action of regio-specific lipases. For example, the 1, 3-regio-specific lipase from Rhizomucor miehei is used on the industrial scale to replace palmitic acid moities of palm oil with stearic acid yielding stearic–palmitic–stearic triacylglycerol, which is a cocoa butter substitute. Similarly, enrichment of oils with highly unsaturated fatty acids can be made by lipase-mediated

transesterification reactions to produce nutraceuticals. Lipases are also used to assist in the extraction of fats and oils and for the development of cheese flavor. Owing to their stability in organic solvents, lack of cofactor requirement, and range of substrate selectivities, lipases are the most utilized enzymes in mediating reactions in organic synthesis with hundreds of research papers on the subject. There is an increasing adaptation of the versatile microbial lipases in the large scale synthesis of fine chemicals, in various organic synthesis processes including the preparation of chiral compounds in enantiomerically pure form.

Enzymes in the fish and meat processing

Proteinases, either indigenous (cathepsin) or those obtained from plants and microorganisms, are used in the meat and fish industries to tenderize meat and solubilize fish products. Plant proteinases include papain from papaya, ficin from figs, and bromelain from pineapples. Microbial enzymes include fungal (A. oryzae, A. niger) and bacterial (B. subtilis, B. lichiniformis) proteases. Tenderization of meat can be achieved by keeping the rapidly chilled meat at 1–2^{o}C to allow proteolysis by indigenous enzymes (cathepsins and the $Ca2^{+}$- dependent neutral protease). Current practice in

the United States favors immersion of meat cuts in concentrated enzyme solutions, followed by vacuum packaging and refrigeration for up to 3 weeks. Enzymes are also used to facilitate separation of hemoglobin from blood proteins and removal of meat from bones. For the preparation of pet food, minced meat or meat by-products are hydrolyzed by proteases to produce a liquid meat digest or a slurry with a much lower viscosity. Pets like the savory flavor generated by peptides and amino acids are produced from the enzymatic hydrolysis of meat. Fish protein concentrates are generally prepared by treating ground fish parts with a protease. After hydrolysis, the bones and scales are removed by screening, and the mixture of solubles and undigested fish solids is dried or separated by centrifugation. The yield of solubles, mainly amino acids and peptides, is generally 60–70% of the initial fish solids. Enzymatic treatment of fish stick water (press water obtained in fish meal manufacture) is performed with a microbial protease (from B. lichiniformis) before concentration by evaporation. This reduces its viscosity to 20–50% which helps to increase the final solid contents by 55–73% under industrial conditions. Thus, the use of enzymes substantially saves in drying costs. Enzymes (xanthin oxidase, catalase, nucleoside phosphorylase and nucleotidase) can also be used for testing the quality and freshness of fish.

Enzymes in starch conversion

Feedstocks containing starch include most of the cereal grains (corn, sorghum, barley) and tuberous crops such as potatoes. Starch contains about 15–30% amylose and 70–85% amylopectin. Amylose (MW about 300 000) is a long linear polymer of alpha-1,4-linked glucose residues, whereas, amylopectin is a branched polymer having both alpha-1,4 and alpha-1,6 linkages. The branched chains may contain from 20 to 30 glucose units. Enzymes have largely replaced the use of strong acid and high temperature to break down starchy materials. Three types of enzymes are involved in starch bioconversion: endo-amylase, exo-amylases, and debranching enzymes. Alpha-Amylase hydrolyzes internal Alpha-1,4-glycosidic bonds of starch at random in an endo-fashion producing malto-oligosaccharides of varying chain lengths. It cannot act on alpha-1,6 linkages. The enzyme is produced by bacteria such as Bacillus lichiniformis, Bacillus subtilis, and Bacillus amyloliquefaciens and fungi such as Aspergillus oryzae. Glucoamylase cleaves glucose units from the non reducing end of starch and it can hydrolyze both alpha-1,4 and alpha-1,6 linkages of starch. It is, however, slower in hydrolyzing alpha-1,6 linkages. Glucoamylase is produced by various genera of fungi

such as Endomycopsis, Aspergillus, Penicillium, Rhizopus, and Mucor. Beta-Amylase hydrolyzes the alpha-1,4-glycosidic bonds in starch from the nonreducing ends, generating maltose. The enzyme is unable to bypass the alpha-1,6 linkages and leaves dextrins, known as beta-limit dextrins. Beta-Amylase is produced by a number of microorganisms including Bacillus megaterium, Bacillus cereus, Bacillus polymyxa, Thermoanaerobacter thermosulfurogenes and Pseudomonas sp. Pullulanase (pullulan alpha-1,6-glucanohydrolase) or isoamylase (glycogen alpha-1,6-glucanohydrolase) cleaves the alpha-1,6-linked branch points of starch and produces linear amylosaccharides of varying lengths. Pullulanase is produced by Aerobacter aerogenes and isoamylase is produced by Pseudomonas amyloderamosa. The dual-function enzyme, amylopullulanase, hydrolyzes both alpha-1,4 and alpha-1,6 linkages of starch and generates DP2–DP4 (DP, degree of polymerization) as products. The enzyme has potential for use in both liquefaction and saccharification of starch. Amylopullulanase is produced by various anaerobic as well as aerobic bacterial species.

Production of glucose syrup

D-Glucose (dextrose) can readily be produced from starch by acid hydrolysis. However, this process has disadvantages such

as a low yield of glucose (85%), formation of undesirable bitter sugar (gentiobiose) and the inevitable formation of salt (from subsequent neutralization with alkali) and coloring materials. With the discovery and development of thermostable alpha-amylase from Bacillus licheniformis, an enzymatic process has replaced the acid hydrolysis process. Enzymatic production of glucose from starch usually involves two essential and distinct steps: liquefaction and saccharification. First, an aqueous slurry of corn starch is gelatinized and partially hydrolyzed by a highly thermostable alpha-amylase to a dextrose equivalent (DE) of 10–15. The optimal pH for the reaction is 6.0–6.5 and $Ca2^{+}$ (1 m mol l^{-1}) is required. $Ca2^{+}$ is a structural factor needed by alpha-amylase for maintaining protein stability and it does not participate in catalysis as an enzyme cofactor. DNA technology has been used to modify the $Ca2^{+}$ binding site to improve binding affinity and lower $Ca2^{+}$ levels needed for stabilization. During liquefaction, alpha-amylase hydrolyzes alpha-1,4 linkages at random, lowering the viscosity of the gelatinized starch. Liquefied and partially hydrolyzed starches are known as maltodextrins and are widely used in the food industry as thickeners. In the saccharification step, temperature of the reaction is brought to 55–60 ^{o} C, pH is lowered to 4.0–5.0 and glucoamylase is added. Long reaction

times of 24–72 h are required depending on the enzyme dose and the percent of glucose desired in the product. Efficiency of saccharification with glucoamylase can be improved by adding pullulanase or isoamylase. This supplemental enzyme addition increases the glucose yield (about 2%), lowers the saccharification time from 72 to 48 h, allows for increased substrate concentrations (to 40%, DS) and lowers the use of glucoamylase by up to 50%. Thus, a glucose yield of 95–97.5% is achieved by including a starch-debranching enzyme with glucoamylase. At high glucose concentrations, glucose molecules can polymerize in a reaction called reversion, forming unwanted byproducts such as maltose, isomaltose and higher saccharides that decrease the glucose yield and purity. The polymerization reaction can be catalyzed by glucoamylase itself or by another enzyme called transglucosidase, which is often present in crude glucoamylase preparations. Typically, glucose syrups (DE 97–98) having 96% glucose contain 2–3% disaccharides (maltose and isomaltose) and 1–2% higher saccharides.

Production of High-fructose corn syrups (HFCSs)

Glucose isomerase (also known as xylose isomerase,) is an example of the highly successful application of enzyme biotechnology to an industrial process that has no commercially viable route

through conventional chemistry. Chemical isomerization of glucose to fructose at high pH and high temperature leads to undesirable side products, some of which are colored and have off flavors. Enzyme catalyzed isomerization (at moderate pH and temperature) does not form undesired side products. Produced intracellularly by Streptomyces, Bacillus, Arthobacter and Actinoplanes species, glucose isomerase is used to convert glucose into fructose to exploit the greater sweetness of fructose over glucose and sucrose. High-fructose corn syrups (HFCSs) are prepared by enzymatic isomerization of glucose syrups in column reactors containing immobilized enzyme. Reactors are run at pH 7.5–8.0 and 55–60°C. $Mg2^{+}$ is usually added as a cofactor and stabilizer of the isomerase and it also alleviates inhibition of the enzyme by $Ca2^{+}$ (present due to some carryover from its use in starch liquefaction with alpha-amylase). Immobilized glucose isomerase has a stability half-life of around 200 days in industrial practice. At equilibrium, the interconversion reaction of glucose and fructose modestly favors glucose over fructose at reactor temperatures (55–60°C). Therefore, at most, glucose isomerase can produce HFCS containing 42% fructose from glucose syrups (95% glucose). Two grades of HFCS are available in the market – HFCS-42 and HFCS-55 which contain 42% and 55% fructose,

respectively, based on dry substance. HFCS-42 syrup is fractionated in a chromatographic column to yield 90– 95% fructose syrup, which can be blended back with HFCS-42 to make HFCS-55 (which matches the sweetness of sucrose on an equivalent mass basis) for use in soft drinks. DNA technology has been used to introduce amino acid substitutions in glucose isomerase that enhance its thermal stability. Further increases in the thermal stability of glucose isomerase are desirable because the equilibrium constant (fructose/glucose) increases with temperature and if the enzyme could withstand 90°C, then it could produce HFCS-55 without necessitating a chromatographic enrichment step.

Production of high-maltose conversion syrups

Various maltose-containing syrups are used in the brewing, baking, soft drink, canning, confectionery and other food industries. There are three types of maltose-containing syrups: high-maltose syrup (DE 35–50, 45–60% maltose, 10–25% maltotriose, 0.5–3% glucose), extra high-maltose syrup (DE 45–60, 70–85% maltose, 8–21% maltotriose, 1.5–2% glucose) and high conversion syrup (DE 60–70, 30–47% maltose, 35–43% glucose, 8–15% maltotriose). Production of these syrups from starch generally involves liquefaction and saccharification, as in

the production of glucose. However, in this process, the liquefaction reaction is terminated when the DE reaches about 5–10, since a low DE value increases the potential for attaining high maltose content. Depending on the maltose content of the syrup desired, saccharification is generally performed by using a maltogenic amylase such as beta-amylase, beta-amylase with pullulanase or isoamylase, or a fungal alpha-amylase at pH 5.0–5.5 and 50–55 °C. High conversion syrups are produced from liquefied starch (DE, generally 40) by saccharification with a carefully balanced mixture of beta-amylase or fungal alpha-amylase and glucoamylase. After partial saccharification, the syrup is heated to destroy the enzyme action and prevent further glucose formation.

Enzymes in beer and wine production

Beer brewing involves malting the barley in a malt house followed by the preparation and fermentation of the wort in the brewery, while wine making requires the extraction of juice from grapes and subsequent fermentation of the juice by yeast. Enzyme technology plays a central role in both these processes. The addition of exogenous glucanases and related polysaccharidases are known to improve not only the beer and wine qualities, but also their overall production efficiency.

Beer brewing: This technology is based on the action of enzymes

activated during malting and fermentation. Malting of barley depends on seed germination, which initiates the biosynthesis and activation of alpha- and beta-amylases, carboxypeptidase and beta-glucanase that hydrolyse the seed reserve. All these enzymes should act in synergy under optimal conditions to produce high quality malt. Nevertheless, many breweries end up using un-malted or poor quality barley, due to seasonal variations, different cultivars or poor harvest, which contains low levels of endogenous beta-glucanase activity. The problem associated with the use of such un-malted or poor quality barley and other cereals in combination with malt is the presence of 6–10% nonstarch polysaccharide (NSP), mainly a soluble beta-glucan. This forms gels during the brewing process and leads to poor filtration of the wort, slow run-off times, low extract yields and /or the development of haze in the final product. To overcome these problems, microbial b-glucanases, which hydrolyse beta-glucan and reduce the viscosity of the wort are added either during mashing or primary fermentation. The commonly used beta-glucanases are from Penicillium emersonii, Aspergillus niger, Bacillus subtilis and Trichoderma reese.

Wine production: This is a biotechnological process in which both yeast cells and enzymes play a key role. Three main exogenous

enzymes used in wine production are pectinases, beta-glucanases and hemicellulases. The main benefits of using these three enzymes during wine making include: (1) better skin maceration and improved colour extraction; (2) easy must clarification and filtration; and (3) improved wine quality and stability. Recently, a fourth enzyme, beta-glucosidase has attracted considerable attention in the wine industry because of its ability to improve the aroma of wines by modifying naturally present, glycosylated precursors. The first microbial enzyme used in the wine industry was a commercial pectinase from Aspergillus, which contained varying amounts of pectin esterase, polygalacturonase, pectin lyase and small amounts of hemicellulase. Addition of pectinase, while crushing grapes or to the wine must, improves juice extraction, reduces the clarification time and increases the terpene content of wine. Also, pectinase preparations with high pectin lyase and low pectin methyl esterase activities are preferred to minimise the methanol released from methylated polygalacturonic acid during wine production.

Enzymes involved in feed processing

Several enzymes like phytase, amylase, beta-glucanase and xylanase are added to the cereal-based diets of such monogastrics to increase the utilization of dietary phosphorous, starch, beta-

glucans and arabinoxylans, respectively. It has been found that supplementing dairy cow and feedlot cattle diets with fiber degrading enzymes has significant potential to improve feed utilization and animal performance. Ruminant feed enzyme additives, primarily xylanases and cellulases, are concentrated extracts resulting from bacterial or fungal fermentations that have specific enzymatic activities. Improvements in animal performance due to the use of enzyme additives can be attributed mainly to improvements in ruminal fiber digestion resulting in increased digestible energy intake. This approach offers exciting possibilities for using enzymes to improve nutrient digestion, utilization, and animal productivity and at the same time reduce animal fecal material and pollution. Spraying enzymes onto feeds just before feeding provides increased management flexibility and bypasses any negative interactions that the ensiling process may have on silage enzyme performance. Treating feeds with enzymes in this manner may improve digestibility via a number of different mechanisms including, direct hydrolysis, improvements in palatability, changes in gut viscosity, complementary actions with ruminal enzymes and changes in the site of digestion. Protease enzymes may improve the digestion of cereal grains, because starch digestion is partially a function of the protein– starch matrix within the seed. Treating

steam flaked sorghum with an enzyme mixture improved weight gain and feed efficiency in steers by about 10%. Fiber degrading enzymes may also help to improve the digestion of cereal grains with fibrous seed coats. Cellulase/xylanase enzymes sprayed onto a barley and barley silage diet improved weight gain and feed efficiency in steers.