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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Therapeutic Application of Pineapple Protease (Bromelain): A Review

Bitange Nipa Tochi[1,2], Zhang Wang[1], Shi - Ying Xu[1] and Wenbin Zhang[1]
[1]Department of Food Science and Engineering, School of Food Science and Technology, Jiangnan University, Wuxi 214122, PR China
[2]Pwani University College - Formerly Kilifi Institute of Agriculture, P.O. Box: 195, Kilifi, Kenya, East Africa

Abstract: Bromelain (EC 3.4.22.32) is a crude extract from the pineapple (*Ananas comosus*) plant that contains, among other components various closely related proteinases (stem bromelain, fruit bromelain, comosain and ananain) demonstrating both *in vitro* and *in vivo* several therapeutic properties including malignant cell growth, thrombus formation, inflammation, control of diarrhoea, dermatological and skin debridement among others. Bromelain also contains peroxidase, acid phosphatase, several protease inhibitors and organically bound calcium and remains stable over a wide range of pH 2 to 9. Available evidence indicates bromelain is well absorbed orally with its therapeutic effects being enhanced in a dose dependent manner. It has been demonstrated to be safe and an effective food supplement. However, all the mechanisms of its action remain unresolved.

Key words: Bromelain, pineapple, proteinases, inflammation

Introduction

Pineapple (*Ananas comosus*) native to Central and South America, is grown in several tropical and sub-tropical countries including Hawaii, India, China, Kenya, South Africa, Malaysia, the Philippines and Thailand.

It has been used as a medicinal plant in several native cultures and bromelain has been chemically known since 1876 (Peckoldt *et al*., In: Taussig, 1988). Bromelain obtained from the stems of the pineapple plant contains all the soluble components of the pineapple stem in their original properties, which may involve malignant cell growth, thrombus formation, inflammation, control of diarrhoea, dermatological and skin debridement (Cohen, 1964; Taussig and Batkin, 1988; Kelly, 1996; Maurer, 2001).

Heinecke and Gortner (1957) found that bromelain concentrations is high in pineapple stems necessitating its extraction and use as a phytomedical compound because unlike the pineapple fruit which is normally used as food, the stems are a waste by-product and thus inexpensive.

The main proteolytic constituents contained in pharmacological preparations or food supplements of bromelain (stem bromelain, fruit bromelain and ananain) are also present in the pineapple fruit (Hale *et al*., 2005). Bromelain's primary component is a Sulfhydryl proteolytic fraction. Bromelain also contains a Peroxidase, acid Phosphatase, several protease inhibitors and organically bound calcium (Kelly, 1996). Bromelain activity is stable over a wide pH range (Cohen, 1964; Taussig and Batkin, 1988; Kelly, 1996; Maurer, 2001; Heinecke and Gortner, 1957; Hale *et al*., 2005; Mynott *et al*., 1999; Cooreman *et al*., 1976). Therefore it may not be necessary to enteric-protect the protease from acid conditions in the stomach. However, it may be necessary to protect the enzyme from digestion by acid proteases in the gut. It may be administered with a buffering agent, for example bicarbonate or in water or in a solution containing nutrients to assist with absorption of fluid and nutrients (Mynott *et al*., 1999).

Several studies have been carried out and results generated indicate bromelain has useful phytomedical applications. However, these results are yet to be amalgamated and critically compared so as to chat the way forward as to whether bromelain will gain wide acceptance as a phytomedical supplement. The purpose of the present paper is to highlight some relevant contributions regarding bromelain's phytomedical applications that have been reported in recent times.

Anti - inflammatory agent: Botanicals such as *Ananas comosus* (Pineapple) and their extracts (bromelain) have been used clinically as anti-inflammatory agents in rheumatoid arthritis, soft tissue injuries, colonic inflammation, chronic pain and asthma (Taussig and Batkin, 1988; Kelly, 1996; Maurer, 2001; Cooreman *et al*., 1976; Izaka *et al*., 1972; Hale *et al*., 2005; Jaber, 2002) and are currently in use as anti-inflammatory agents (Ammon, 2002; Lemay *et al*., 2004; Darshan and Doreswamy, 2004).

The major mechanism of action of bromelain appears to be proteolytic in nature, although evidence also suggests an immunomodulatory and hormone like activity acting via intracellular signalling pathways. *In vitro* studies have shown that bromelain can inhibit pre-incubated with medium alone (PMA) - induced T cell production of the Th_2 cytokine IL - 4 and to a lesser degree the Th_1 cytokines IL-2 and induced interferon-gamma (IFN-γ) via modulation of the extracellular

regulated kinase-2 (ERK-2) intracellular signalling pathway (Mynott *et al*., 1999). Bromelain has also been shown to reduce cell surface receptors such as hyaluronan receptor CD44, which is associated with leukocyte migration and induction of proinflammatory mediators (Engwerda *et al*., 2001; Eckert *et al*., 1999; Hale *et al*., 2002). Manhart *et al*. (2002) have shown bromelain to significantly reduce $CD4^+$ T lymphocytes, which are primary effectors in animal models of inflammation.

Beneficial effects of bromelain have been suggested or proven in a variety of inflammatory disease and animal models of inflammation. These include immunologically mediated arteriosclerosis in rat aortic allografts (Gaciong *et al*., 1996), the experimental allergic encephalomyelitis (EAE) model for the human autoimmune disease multiple sclerosis (Targoni *et al*., 1999; Hale *et al*., 2005), IgE- mediated perennial allergic rhinitis (Thornhill and Kelly, 2000) and collagen-induced arthritis in the rat (Rovenska *et al*., 2001). In their study on bromelain's anti-inflammatory effects in an ovalbumin - induced murine model of allergic airway disease (AAD), Secor *et al*. (2005) observed that bromelain demonstrated both anti-inflammatory and immunomodulatory effects. In this particular study, they found that bromelain treatment significantly reduced the primary outcomes of murine AAD: Total bronchoalveolar lavage (BAL) leukocytes (eosinophilis and lymphocytes), IL-13, $CD4^+$ T cells, $CD8^+$ T cells and $CD4^+CD25^+$ T cells, while also altering the $CD4^+/CD8^+$ ratio. These findings indicate that systemic bromelain treatment reduces an allergen induced localized airway inflammatory process.

Kane and Goldberg (2000) gave a description of two patients suffering from ulcerative colitis (UC) that was refractory to conventional treatment, who rapidly entered and remained in clinical and endoscopic remission after self treatment with oral bromelain obtained from a healthy food store. Studies by Hale (2004) show that daily treatment with 5mg of oral bromelain significantly decreased spontaneous colon inflammation in $IL\text{-}10^{-1-}$ mice. They further did show that anti-inflammatory activity of bromelain is dependent on its proteolytic activity.

Wen *et al*. (2006) while studying the effect of bromelain on postoperative defecation in rats, supports Hale's (2004) findings that proteolytic activity of bromelain in the colonic micro-environment is not only responsible for its anti-inflammatory activity but may be involved in the improvement of post operative ileus. That orally administered bromelain retains its proteolytic activity, was previously documented only in the small intestine of pigs (Mynott *et al*., 1996; Chandler and Mynott, 1998). The bromelain used in this study was enterically protected. In his studies however, Hale (2004) showed that oral bromelain retains its proteolytic activity throughout the entire gastrointestinal tract of mice in the absence of encapsulation or other classic enteric protection techniques. These results could explain earlier reports that bromelain decreased intestinal inflammation in human with UC (Kane and Goldberg, 2000).

Walker *et al*. (2002), in their pilot study while investigating the effect of bromelain on acute knee pain, reported significant improvement after a month's intervention. These results were consistent with earlier reports of bromelain supplementation (Uhlig, 1981; Vogler, 1988; Lotti *et al*., 1993) even though they could not be compared directly. Meanwhile Akhtar *et al*. (2004) in their study where they assessed the efficacy of an oral enzyme combination (ERC: Enzyme-rutin combination which contains rutin and enzymes bromelain and trypsin) versus diclofenac (a non-steroidal anti-inflammatory drug-NSAID) among patients with knee osteoarthritis (OA) in a double blind randomized version, found ERC to be as equally efficacious to diclofenac. These results are consistent with those reported earlier by (Vogler, 1988; Klein and Kullich, 2000; Tilwe *et al*., 2001). More so, unlike diclofenac, which exhibits inherent toxicities, ERC has a well known superior safety and tolerable profile (Akhtar *et al*., 2004).

Bromelain as an anti-tumour agent: Pharmacological agents with modulation of anti-inflammatory, proteolytic, platelet aggregation inhibition and prostaglandin synthesis have been considered to be beneficial in regulating tumour growth and its metastasis (Batkin *et al*., 1985; Honn, 1983; Sato *et al*., 1983). Bromelain, with similar regulating actions, has shown protective properties on tumour cell growth retardation and lung metastasis (Batkin *et al*., 1985; Batkin *et al*., 1988a; Batkin *et al*., 1988b; Taussig and Batkin, 1988).

Batkin *et al*. (1988b) while studying the antimetastatic effect of bromelain with or without its proteolytic and anticoagulant activities in the animal model of Lewis lung carcinoma, reported significant reduction in total number of metastasis in both active and inactive bromelain as compared to control groups. This phenomenon had been reported earlier by Batkin *et al*. (1985) whose study of three cell lines was done *in vitro*. In both studies, they conclude that bromelain could be having other pharmacological entities besides its recognized proteolytic anticoagulant functions.

Recently, study results reported by researchers at the Queensland Institute of Medical Research-QIMR (QIMR, 2005), give a window of hope for this phenomenon. While studying bromelain, researchers at QIMR reported the discovery of two proteins they named CCS and CCZ and found that they could block growth of a broad range of tumour cells including breast, lung, colon, ovarian and melanoma. However, the study is on going and these results are not reliable at the moment. Batkin *et al*. (1985 and 1988a) noted that *in vitro* Lewis lung cancer cell growth retardation was a necessary correlate to antimetastatic activity. In this regard therefore,

peroxidase and proteolytic anticoagulant activities may not be relevant features of bromelain's antimetastatic potential.

Maurer *et al.* (1988) found that bromelain may induce differentiation of leukemic cells *in vitro* and proposed this phenomenon as a possible mechanism of action. In their studies, Grabowska *et al.* (1997) found that B16F10 mouse melanoma cells, pre-incubated *in vitro* with bromelain, significantly reduced lung metastatic tumour weight to about three times. However, no survival benefit was seen. Furthermore bromelain diminished the capacity of these cells to migrate through an extracellular matrix layer in an in *vitro* invasion assay and inhibited the growth of tumour cells in a concentration dependent manner, whereas the anti-proliferation effect did not correlate with the proteolytic activity. Earlier studies by Goldstein *et al.* (1975) and Taussig and Goldstein (1976) reported that bromelain feeding enhanced the resistance of mice to the harmful effect of UV (Ultra Violet) irradiation. It took twice as long for the bromelain fed group to develop pre-cancerous lesions as compared to the control group. Finally, human platelets pre-treated *in vitro* with bromelain lost their capacity to stimulate the invasiveness of several metastatic tumour cells in the *in vitro* invasion assay. Meanwhile it has been shown that metastasized cells, while migrating through the vessels, carry CD44 adhesion molecules on their surface by which they adhere to endothelial cells via the ligand hyaluron. Bromelain preferentially cleaves off CD44 molecules by virtue of its proteolytic activity, thus inhibiting the first steps of the metastatic process (Eckert *et al.*, 1999; Hale *et al.*, 2002; Hale and Haynes, 1992).

Maurer (2001) noted that metastasized tumour cells carry the receptor (uPAR) for urokinase plasminogen activator (uPA), which generates plasmin from plasminogen. Plasmin degrades the extracellular matrix (ECM), composed of collagen type IV, laminin and fibronectin. Tumour cells also secrete matrix metalloproteinases (MMPs), enabling the malignant cells to invade through the ECM. Bromelain diminishes uPAR expression and uPA activity, thus inhibiting the invasion step of metastasis. Maurer further notes that interactions between tumour cells and platelets take place on different levels i.e. intravasal distribution, adhesion on endothelial cells, invasion and extravasation. Platelets he notes, directly bind to tumour cells, a process promoted by the release of factors such as platelet factor 4, thrombospondin, thrombin and gelatinase A from platelets, which facilitate thrombus formation. Apart from this, transforming growth factor- β (TGF-β), produced by both platelets and tumour cells, plays an important role: it induces the synthesis of ECM proteins and stimulates the activity of uPA, MMPs and angiogenesis. Thus, disturbance of the blood coagulation system may lead to the formation of thrombi by aggregating platelets and tumour cells. Bromelain is capable of inhibiting both platelet aggregation *in vitro* and *in vivo*, as well as platelet-stimulated invasiveness of tumour cells. Thus what was described and used as folk medicine by the natives of the tropics over a Century ago as over time been confirmed to have pharmaceutical applications. However, more research is required to determine the structure and characteristics of the two compounds (CCS and CCZ) that were reported by researchers at the QIMR (QIMR, 2005).

Bromelain promotes debridement of burns: Burns are characterized by formation of an eschar, which is made up of burned and traumatized tissue. The eschar not only hinders accurate diagnosis of the burn's depth but also serves as a medium for bacterial growth and therefore a source of infection, contamination and sepsis of the injury and to the neighbouring originally undamaged tissues (Rosenberg *et al.*, 2004). Rapid debridement considerably reduces morbidity and mortality of severely burned patients. It permits early skin grafting and lessens the problems of infection, contamination and sepsis thus abbreviating the convalescence period (Maurer, 2001; Sheridan *et al.*, 1994; Sheridan *et al.*, 1998; Prasanna *et al.*, 1994; Monafo, 1974; Nada *et al.*, 1998).

While surgical debridement is non-selective, chemical debridement removes only the burned denatured skin (Maurer, 2001; Sheridan *et al.*, 1994; Janzekovic, 1970; Salisbury, 1990; Miller *et al.*, 1992). Furthermore, surgical excision is painful and exposes patients to the risks of repeated anaesthesia and significant bleeding. Enzymatic debridement has been suggested with experimental runs giving positive results. Topical bromelain (35% in a lipid base) was reported to achieve complete debridement on experimental burns in rats in about 2 days, as compared with collagenase, which required about 10 days, with no side effects or damage to adjacent burned tissue (Klaue *et al.*, 1979). When topical bromelain was used for frostbite eschar removal, no debridement other than of superficial eschar layers was noted; after two topical applications of bromelain, frostbite injuries remained unaffected (Ahle and Hamlet, 1987).

Rosenberg *et al.* (2004), reported complete debridement of the eschar after only one to two brief applications with minimal side effects and no blood loss. In the same study, no specific debridase (a bromelain derived debriding agent) related morbidity or mortality was noted. However, due to incomplete data in a large number of subjects, inaccuracies may have been possible thus calling for more controlled studies to assess the safety and efficacy of a proteolytic enzyme for enzymatic skin debridement.

Effects of bromelain on diarrhoea: Diarrhoea is a major cause of illness and death in children and young animals (Cravioto *et al.*, 1988; Smith and Lingood, 1982;

Roselli *et al.*, 2007). Vibro cholerae and enterotoxigenic Escherichia coli (ETEC) are two important microorganisms that cause diarrhoea Levine *et al.* (1983). ETEC produces one or both of a heat-labile (LT) and/or heat stable enterotoxin (either STa or STb) and V.cholerae liberate cholera toxin (CT) (Mynott *et al.*, 1997). To contain this problem, drugs such as chlorpromazine, nicotinic acid, loperamide and berberine sulfate have been used in animal models to inhibit secretion by CT and LT (Guandalini *et al.*, 1984; Holmgren *et al.*, 1978; Turjman *et al.*, 1978). Berberine, chlorpromazine and indomethacin also reduce secretion induced by STa (Greenberg *et al.*, 1980; Abbey and Knoop, 1979; Guandalini *et al.*, 1987). Despite the efficacy of these antisecretory compounds in animals, none are routinely available for use in children and adults because of adverse side effects or the large doses required for efficacy (Chandler and Mynott, 1998). Over time, oral rehydration therapy which has a significant impact on morbidity and mortality of patients with acute infectious diarrhoea has been used. However, oral rehydration therapy does not interfere with the secretory process nor diminish diarrhoea (Field, 1981). Bromelain has been demonstrated to have anti-diarrhoea activity (Chandler and Mynott, 1998; Thomson *et al.*, 2001).

Studies by Mynott *et al.* (1997) have reported stem bromelain to show antisecretory properties. Using a rabbit ileum mounted in using chambers, they showed that bromelain could prevent net changes in short-circuit current (Isc) and therefore, fluid secretion mediated by secretagogues that act through cAMP (cyclic-3, 5-adenosine monophosphate), cGMP (cyclic-3, 5-guanosine monophosphate) and calcium-dependent signalling pathways. Because most toxins that cause diarrhoea activate one of these pathways, bromelain would be expected to be an effective anti diarrhoea nutraceutical drug.

The efficacy of bromelain in this study was 62% in preventing LT induced secretion, 51% effective against CT and 35% effective against STa. Bromelain also prevented secretory changes caused by prostaglandin E_2, theophylline, calcium-ionophore A23187, 8-Br-cAMP (8-bromocyclic-3, 5-adenosine monophosphate) and 8-Br-cGMP (8- bromocyclic-3, 5-guanosine mono-phosphate), well known intracellular mediators of ion secretion. The efficacy of bromelain was reported not caused by reduced tissue viability resulting from its proteolytic effects on enterocytes, indicated by experiments measuring uptake of nutrients into intestinal cells and experiments measuring short circuit responses to glucose. Meanwhile studies by Roselli *et al.* (2007) on the effect of different plant extracts and natural substances (PENS) against membrane damage induced by ETEC in pig intestinal cells showed bromelain to be among those with protective effect.

Bromelain improves decrease in defecation in ileus condition: Postoperative gastrointestinal dysmotility (ileus) is a common consequence of abdominal surgery causing significant patient discomfort (nausea, vomiting, abdominal distension and inability to eat or defecate), and often leads to more serious problems (acute gastric dilatation, aspiration, respiratory compromise, cardiac arrhythmia and perforation). Due to limited therapy specific for this procedure, ileus remains an important clinical problem (Wen *et al.*, 2006).

In the US, bromelain is sold in health food stores as a nutritional supplement to promote digestive health and as an anti-inflammatory medication for horses (Hale, 2004). It has been used successfully as a digestive enzyme following pancreatectomy in cases of exocrine pancreas insufficiency and in other intestinal disorders (Knill-Jones *et al.*, 1970). Recently, it was reported that stool discharge improved in some Japanese patients concomitantly suffering from haemorrhoids and constipation after using bromelain {private communication in (Wen *et al.*, 2006). This in essence suggests that bromelain may improve intestinal propulsive motility.

In another study, the combination of ox bile, pancreatin and bromelain was shown to be effective in lowering stool fat excretion in patients with pancreatic steatorrhoea. In addition, this combination resulted in a gain in weight in most cases as well as enhanced subjective feeling of well being. Symptomatic improvement was also noted in relation to pain, flatulence and stool frequency (Balakrishnan *et al.*, 1981). In a recent study, Wen *et al.* (2006) did show that treatment with 500 mg/kg bromelain significantly increased wet weight and water content of faecal pellets to near normal levels in postoperative rats. The results suggest that bromelain may play an important role in treatment of ileus. In the same study, bromelain treatment was shown to significantly suppress overexpression of colonic iNOS mRNA, accompanied by improvement of decrease in defecation in postoperative rats. It is therefore suggested that modulation of iNOS gene expression is involved in the improvement by bromelain of the decreased defecation in postoperative rats, at least in part, by inhibiting colonic iNOS gene expression probably through $NF\text{-}_kB$ pathway.

Bromelain inhibits thrombus formation: Studies have indicated that bromelain prevents aggregation of human blood platelets *in vivo* and *in vitro*, prevents or minimizes the severity of angina pectoris and transient ischemic attacks (TIA), is useful in the prevention and treatment of thrombosis and thrombophlebitis, may break down cholesterol plaques and exerts a potent fibrinolytic activity (Taussig and Nieper, 1979; Kelly, 1996). Furthermore, it has been suggested that bromelain increases vessels wall permeability to oxygen and nutrients while increasingly thinning blood both of which aid in these conditions (Kelly, 1996).

Heinicke *et al.* (1972) were the first to report that bromelain prevents aggregation of blood platelets. In their study carried among human volunteers with a history of heart attack or stroke or with people having high aggregation values, as well as with health subjects, oral administration of bromelain (160-1000 mg per day) decreased aggregation of blood platelets in all the subjects. Later studies by Nieper (1978), who administered 400-1000 mg per day of bromelain to 14 patients with angina pectoris resulted in the disappearance of symptoms in all patients within 4 to 90 days but reappeared after bromelain administration was discontinued.

Metzig *et al.* (1999) showed that pre-incubation of human platelets with 10ug/ml bromelain completely prevents thrombin induced platelet aggregation *in vitro* and also reduced the adhesion of thrombin stimulated, fluorescently labelled platelets to bovine aorta endothelial cells. Similarly, they reported that oral (60 mg/kg) and intravenous (30 mg/kg) bromelain inhibited *in vivo* thrombus formation in a model of laser-induced thrombosis in rats. The ability of bromelain to influence these conditions could be due to its ability to breakdown fibrinous plaques. Bromelain has been shown to dissolve arteriosclerotic plaque in rabbit aorta *in vivo* and *in vitro* (Taussig and Nieper, 1979). Later, Hale *et al.* (2002) showed that *in vitro* bromelain treatment of leukocytes in whole blood proteolytically altered 14 of 59 leukocyte makers studied. It is important to note that bromelain induced loss of CD41 and CD42a via proteolysis would be expected to decrease platelet function and thus inhibit thrombus formation.

Bromelain gives strong immunogenicity: Bromelain has been shown to remove T-cell CD44 molecules from lymphocytes among other bromelain sensitive molecules (Hale *et al.*, 2002; Eckert *et al.*, 1999; Hale and Haynes, 1992; Roep *et al.*, 2002; Desser *et al.*, 1993). Munzig *et al.* (1994) did show that highly purified bromelain protease F9 reduced the expression of CD44 to about 10 times more than the crude bromelain, achieving about 97% inhibition of CD44 expression. Roep *et al.* (Roep *et al.*, 2002) reported that protease treatment reduced expression of cell surface receptors on T-cells and antigen-presenting cells. Previously, reduction of CD44 expression on lymphocytes of patients with multiple sclerosis during protease therapy had been reported (Munzig *et al.*, 1994; Stauder *et al.*, 1997; Hale and Haynes, 1992).

Roep *et al.* (2002) suggested that the generation of soluble forms of adhesion molecules by proteolytic cleavage could act as an additional benefit for immonomudulatory function of protease treatment. However, they noted that the quality of immune activation plays an important role during chronic autoimmunity. Earlier, animal models for rheumatoid arthritis and Type 1 diabetes protease treatment prevented or delayed the onset of these diseases (Wiest-Ladenburger *et al.*, 1997; Emancipator *et al.*, 1997; White *et al.*, 1991). Later, Hale (2004) unexpectedly found bromelain to exhibit strong immunogenicity following oral dosing. In further studies following this phenomenon, Hale *et al.* (2006) reported that repeated exposure was necessary for development of anti-bromelain antibodies, with exposure period ranging from 3 to 6 weeks on a dose dependent manner.

Bromelain application in dermatological disorders: Bromelain among other fruit extracts from apricots, apples, peaches, pears, papayas, pomegranates, cherries, kiwis, tangerines and oranges have been described to play an important role in treating dermatological disorders (Murad, 2003). Ozlen (1995) has disclosed a cosmetic composition containing at least one alpha-hydroxy acid, salicylic acid and at least one digestive enzyme derived from fruit. Preferably the digestive enzyme is a mixture of bromelain and papain. Bromelain is disclosed as being typically obtained from pineapple and papain is disclosed as being typically obtained from dry papaya latex. The compositions are allegedly useful for treating various cosmetic conditions or dermatological disorders, such as lack of adequate skin firmness, wrinkles and dry skin.

Conclusion: Bromelain being a plant extract, contains various components such as proteinases, peroxidises, phosphatises, protease inhibitors and organically bound calcium whose ratio to each other might vary according to soil composition, climate conditions during plant growth, geographical location where the pineapple was grown, pineapple variety and the process of extraction. These factors might contribute to the variations of bromelain's pharmacological activities.

Proteolytic activity of bromelain has been shown to play only a part in its pharmacological activity while other factors such as immunomodulatory, hormone like properties, fibrinolytic activity and uncharacterised components such as CCS and CCZ complement towards its pharmacological activity. However, there is need for further investigation on the uncharacterised components.

Bromelain's activity remains stable over a wide pH range which explains why its activity has been found to be effective over the entire gastrointestinal tract. Since it is safe and non toxic, there is need to investigate how it can be incorporated in foods. On our view, if successfully incorporated in foods, it could become more acceptable as a nutraceutical product than it now is.

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Effect of Fermentation and Particle Size of Wheat Bran on the Antinutritional Factors and Bread Quality

Eiman G. Hassan[1], Amir M. Awad Alkareem[2] and Abdel Moniem I. Mustafa[3]
[1]Food Processing Research Centre, Shambat, Sudan
[2]Faculty of Agriculture, University of Alzaim Alazhri, Sudan
[3]Faculty of Agriculture, University of Khartoum, Sudan

Abstract: Three local sudanese wheat cultivars, Debeira, El-Nielain and Sasaraib were obtained from Agricultural Research Corporation (harvest season 2002/03). Wheat bran was obtained from a local flour mill in Khartoum North. It was carefully sieved and classified as coarse, medium and fine bran size then fermented. Proximate composition, mineral content and anti-nutritional factors (tannin, phytic acid) were determined for all types of wheat bran. Results indicated that fermentation of wheat bran increased the percentage of crude fiber from 15.67 to 18.67%, 15.67 to 18.00%, 15.00 to 17.67%, protein content from 20.35 to 21.65%, 18.36 to 20.79%, 21.07 to 22.40% for coarse, medium and fine wheat brans, respectively. Carbohydrates percentage increased from 45.09 to 47.4% in fermented coarse wheat bran. Both anti-nutritional factors (tannins and phytic acid) were found to decrease significantly ($P \leq 0.05$) in coarse, medium and fine wheat bran. The tannin content decreased from 0.03 to 0.01, 0.07 to 0.05 and from 0.07 to 0.06 mg catechin/100 gm, respectively. Phytic acid decreased from 626.1 to 572.8, 740.4 to 367.1 and from 795.2 to 301.6 mg/100 gm, respectively. There is no change on the values of Ca and Fe contents of coarse wheat bran after fermentation. Also there was an increase in Ca content of fine and medium wheat bran. Fe content of medium wheat bran decreased from 0.03 to 0.02% but Fe content of fine wheat bran increased from 0.023 to 0.033%. There is a slight decrease in P content of coarse wheat bran after fermentation. The phosphorous content as percentage decreased in fermented medium and fine wheat bran from 0.004 and 0.003% to 0.002 and .002%, respectively. Bread specific volume values of the three cultivars with 10, 15 and 20% fermented wheat bran decreased with increase in the amount of wheat bran. Bread with 10% fermented coarse wheat bran gave the best results for all characteristics tested in organolyptic evaluation.

Key words: Debeira, El-Nielain, Sasaraib, wheat, mineral content

Introduction

Wheat bran, a by-product of flour milling is composed of the pericarp and the outermost tissues of the seed, including the aleurone layer. It constitutes almost 10% of the total weight of wheat milled for flour. On moisture-free basis, bran contains about 17% protein and 70% carbohydrates, about 80% of which is cellulose and hemicellulose. Most of the bran protein and other nutrients are contained in the aleurone cells (Saunders *et al*., 1972). Ranum (2000) found that bran is mainly cellulose with very little gluten, so there is not much it can be used for other than being a source of fibre, but it does contain higher vitamin and mineral contents, so flour made with a higher extraction rate tends to be more nutritious. However, bran also contains higher levels of phytic acid, which makes the minerals less available to the body, particularly when used in a non-fermented food product. Stanyon and Costello (1990) used wheat bran to enhance the nutritional quality of baked products such as cakes, yeast bread and muffins. McRorie *et al*. (2000) reported that consumption of wheat bran in excess of levels in a typical western diet significantly increased stool output.

Malkki (2001) reported that the physiological effects of dietary fiber are usually compared with the intakes or contents of total dietary fiber. Many health-related effects such as cholesterol reduction attenuation of blood glucose and insulin and prolonged satiety, are due to the physical properties of a fiber. Dirar (1993) stated that fermentation is a method of preservation which may destroy undesirable factors in the raw product; the fermented food may have an enhanced nutritional value and digestibility. He further reported that fermented foods have a better flavour than the raw products. Wheat bran is more detrimental to loaf volume of bread and found to increase dough water absorption (Birch and Finney, 1980; Shogren *et al*., 1981; Rogers and Hoseney, 1982; Moder *et al*., 1984; Lai and Hoseney, 1989). Jeltema *et al*. (1983) reported that hemicellulose increases dough water absorption too. Mongeau and Brassard (1986) reported that addition of wheat and maize bran progressively reduced all bread quality characteristics.

Katina *et al*. (2001) reported that in baking, however, addition of wheat bran results in bread with inferior quality, low volume poor crumb structure, poor shelf-life

and a bitter flavour. They added that pre-fermentation of wheat bran with yeast or yeast and lactic acid bacteria improved the loaf volume, crumb structure and shelf-life of bread supplemented with bran. The positive effect of fermentation of bran on bread quality was evident in the changes of protein network structure of the breads. Pre-fermentation of the bran with yeast and lactic acid bacteria had the greatest effect on the structure of starch. Furthermore, Katina *et al*. (2001) revealed that the bread also had added flavour and good homogeneous crumb structure and elasticity of the crumb was excellent. Mustafa *et al*. (2002) reported that fermentation darkens the bran color, which is observed in the bread produced. Further fermentation is noticed to increase the flavour and acidity. The objective of this study was to evaluate the effect of fermentation as a mean of reducing anti-nutritional factors on the different fractions of wheat bran and on bread quality.

Materials and Methods

Cleaning of the three local wheat grains (Debeira, El-Nielain and Sasaraib) was done by aspiration sieving and manual separation of impurities by hand. For obtaining uniform seeds, 2.8 micron sieve was used for removing small grains. A sample of each cultivar was tempered to 13.5% moisture for 24 hours, then milled in Barbender Quadrumat Junior mill (Regulation No. 1) to white flour, the flour was adjusted to 72% extraction rate by adding the right amount of ground and sieved bran of the same wheat (if needed). Each sample was well mixed and placed in air tight plastic container, then stored under appropriate conditions (deep freezer) until used.

Wheat bran was sieved and separated to pass through a special plan sifter (sieve 355, 500 and 710 micron). The throughs were classified as fine bran over 355 micron sieve, over 500 micron sieve were classified as medium bran and the overs of the sieve No. 710 micron were classified as coarse bran. Each bran fraction was well mixed and stored in air-tight plastic container in a deep freezer until used.

Each wheat bran fraction (coarse, medium and fine wheat bran) was mixed with 2% dry yeast and 30 ppm ascorbic acid, then covered with water, well mixed and placed in an incubator at 30°C for 4 hours (incubator RO-8 memmerr made in Western Germany). The bran was spread on a wide tray then placed in an oven (oven Heraeus Type T5050 Fabrik-Nr 8204271) set at 70°C until dry. Each of the samples was packed in polyethylene bags and stored in a deep freezer until used.

The wheat bran fraction (fermented and non-fermented coarse, medium and fine) were mixed with wheat flours (Debeira, El-Nielain and Sasaraib wheat flour) in the percentages 10, 15 and 20%.

Chemical analysis of the wheat bran: The moisture content was determined according to the method of AACC (1983) using Buhler Rapid moisture tester (type MLI-1000). The ash content was measured according to the AOAC method (1990) using muffle furnace (model Tipoforno ZA No. 18203 Gef Ran 1001). AOAC (1984) methods were used for determining protein, fat and crude fiber content. The total carbohydrates were calculated by difference according to Pearson (1976). Determination of minerals (Ca and Fe) was carried according to Pearson (1970) by the Atomic Absorption Spectrum (A.A.S) model G.B.C 932, while phosphorus was determined by the Spectrophotometer (Model CECILCE 1021 1000) series according to Pearson (1970). Tannins were determined by Price *et al*. (1978) techniques using Vanillin-HCl in methanol and 1% vanillin/methanol, while phytic acid by the method of Wheeler and Ferrell (1971).

Bread making procedure: The various wheat flour/wheat bran blends and the control (0.0% wheat bran) were fermented and baked according to the procedure described by Badi *et al*. (1978) modified as follows:

Ingredients	1	2	3	4
Wheat flour	100%	90%	85%	80%
Wheat bran	0	10%	15%	20%
Water	F.W.A +3%	F.W.A +6%	F.W.A +6%	F.W.A +6%
Sugar	2%	2%	2%	2%
Salt	1%	1%	1%	1%
Yeast	1%	1%	1%	1%
Shortening	2%	2%	2%	2%
Improver (Ascorbic Acid)	80 ppm	80 ppm	80 ppm	80 ppm

Where F.W.A = Farinograph water absorption.

The tap-water was used in this process. All ingredients were then weighed and mixed for 5 min. to form a dough in mono-universal laboratory dough mixer at medium speed. The dough was allowed to rest for 10 minutes at room temperature (30°C) and then scaled to three portions of 120 g each. The three dough portions were made into round balls and allowed to rest for another 15 minutes then molded and put into a pan and placed in the fermentation cabinet for final proof between 50 and 60 minutes. Baking was done in Simon Rotary Test Oven at 220°C with steam saturation. Baking time was 10-15 minutes. After one hour, the loaves were weighed in grams and the volumes were measured in ml using the millet seed displacement method (volumeter). Different types of breads were prepared using wheat flour blends with fermented wheat bran (different bran particle sizes and different percentages). Also, whole wheat flour bread was prepared as a second control sample. The loaves were sliced with an electric knife

Table 1: Proximate composition of wheat bran

---------- Wheat bran ----------		Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Fibres (%)	CHO (%) (- fibre)
Non-fermented	Coarse	9.78[a]	20.35[d]	5.82[a]	3.28[c]	15.67[a]	45.09[a]
	Medium	9.70[a]	18.36[e]	5.46[c]	3.26[c]	15.67[a]	47.55[c]
	Fine	9.77[a]	21.07[c]	4.85[e]	4.26[a]	15.00[a]	45.05[e]
Fermented	Coarse	5.07[d]	21.65[b]	5.49[c]	1.73[f]	18.67[a]	47.40a
	Medium	6.80[c]	20.79[c]	5.68[b]	2.78[d]	18.00[a]	45.96[ab]
	Fine	7.70[b]	22.40[a]	4.98[d]	3.77[b]	17.67[a]	43.48[ad]

*Any mean values in the same column having different superscript letters differ significantly (P≤0.05).

Table 2: Effects of fermentation on the antinutritional factors (tannins and phytic acid)

Wheat bran	Tannin (mg catechin/ 100 gm material)	Phytic acid (mg/100 g)
Non-fermented		
Coarse	0.03[d]	626.12[c]
Medium	0.06[b]	740.36[b]
Fine	0.07[a]	795.20[a]
Fermented		
Coarse	0.01[e]	572.79[d]
Medium	0.05[c]	367.13[e]
Fine	0.06[b]	301.63[f]

*Any mean values in the same column having different superscript letters differ significantly (P≤0.05).

and the slices were kept closed in polyethylene bags at room temperature for sensory evaluation in the same day.

Physical characteristics: Different blends were tested for bread making quality. The loaf volume expressed in cubic centimeters was determined by the seed displacement method according to Pyler (1973). The loaf was placed in a container of known volume into which small seeds (millet seeds) were run until the container is full. The volume of seeds displaced by the loaf was considered as the loaf volume. The loaf weight of bread was measured in gm. Specific loaf volume was calculated as loaf volume (cc) divided by loaf weight in grams.

Sensory analysis: A panel of 15 members, composed of males and females, was used to judge the quality of the breads for color, taste, crumb texture, crumb grain and preference using a 10-points hedonic scale as follows:
10-9 as excellent, >9-7 as very good, >7-5 as good, >5-3 as fair and >3-1 as poor.

Statistical analysis: Analysis of variance was carried out according to the SAS (1997) system using 5% level of significance.

Results and Discussion

The proximate composition of non-fermented and fermented wheat bran is presented in Table 1. The fermentation of wheat bran has affected the chemical components of wheat bran. The moisture content of the wheat bran, which was 9.78, 9.70 and 9.77% decreased

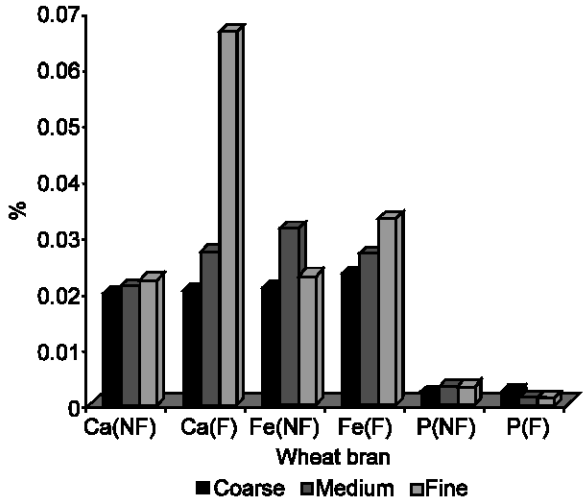

Fig. 1: Effects of fermentation of wheat bran on mineral content

to 5.07, 6.80 and 7.70% on the three fractions (coarse, medium and fine) of wheat bran after fermentation, respectively. This decline of moisture content could be attributed to the drying after fermentation of wheat bran. On the other hand, the fermentation of wheat bran increased the protein percentage from 20.35, 18.36 and 21.07% to 21.65, 20.79 and 22.40%. The fermentation of wheat bran also increased fiber percentage from 15.67, 15.67 and 15.00% to 18.67, 18.00 and 17.67%, respectively for all fractions of wheat bran. The increase in protein and fiber contents was mainly due to the yeast reproduction during fermentation.
The fat percentage of wheat bran decreased from 3.28, 3.26 and 4.26% to 1.73, 2.78 and 3.77%, respectively. The ash percentage of coarse wheat bran decreased from 5.82 to 5.49% and there is a slight increase in the fat percentage of medium and fine bran from 5.46, 4.85 to 5.68 and 4.98%, respectively. However, the fermentation of wheat bran increased the carbohydrates percentage from 45.09% in coarse wheat bran to 47.4%
The protein percentage of non-fermented wheat bran is almost similar to that reported by Ellis (1981). The result of moisture and fat percentages were in good agreement with the results reported by Sid Ahmed (2003).
Tannins content of wheat bran are shown in Table 2. Tannins content of wheat bran is almost similar to that

Table 3: Bread specific volume

Type of bread	Loaf weight	Loaf volume	Loaf specific volume
Sasarib flour+0% fermented bran	107.4^{def}	441.7^{a}	4.11^{a}
Sasarib flour+10% F.C.B	106.6^{efgh}	381.7^{bc}	3.58^{c}
Sasarib flour+15% F.C.B	100.7^{m}	346.7^{de}	3.44^{d}
Sasarib flour+20% F.C.B	101.6^{lm}	313.3^{gh}	3.08^{fg}
Sasarib flour+10% F.M.B	105.0^{j}	391.7^{b}	3.73^{b}
Sasarib flour+15% F.M.B	105.3^{hi}	333.3^{ef}	3.17^{ef}
Sasarib flour+20% F.M.B	103.0^{k}	305.0^{ghijk}	2.96^{ghi}
Sasarib flour+10% F.F.B	109.1^{abc}	391.7^{b}	3.59^{c}
Sasarib flour+15% F.F.B	107.6^{def}	340.0^{de}	3.16^{ef}
Sasarib flour+20%F.F.B	109.6^{a}	310.0^{ghi}	2.83^{ij}
El-Nielain flour+0% fermented bran	106.8^{defg}	353.3^{d}	3.31^{de}
El-Nielain flour+10% F.C.B	101.0^{m}	301.7^{hijk}	2.99^{gh}
El-Nielain flour+15% F.C.B	102.7^{kl}	288.3^{k}	2.81^{ij}
El-Nielain flour+20% F.C.B	109.3^{ab}	253.3^{l}	2.32^{l}
El-Nielain flour+10% F.M.B	103.6^{k}	291.7^{k}	2.82^{ij}
El-Nielain flour+15% F.M.B	105.7^{ghi}	265.0^{l}	2.51^{k}
El-Nielain flour+20% F.M.B	104.5^{ij}	220.0^{m}	2.11^{m}
El-Nielain flour+10% F.F.B	107.3^{def}	310.0^{ghi}	2.89^{hij}
El-Nielain flour+15% F.F.B	105.5^{ghi}	308.3^{ghij}	2.92^{hi}
El-Nielain flour+20%F.F.B	107.2^{def}	268.3^{l}	2.50^{k}
Debeira flour+0% fermented bran	107.9^{cde}	438.3^{a}	4.06^{a}
Debeira flour+10% F.C.B	105.1^{ij}	375.0^{c}	3.57c
Debeira flour+15% F.C.B	103.4^{k}	341.7^{de}	3.30^{de}
Debeira flour+20% F.C.B	106.4^{fgh}	293.3^{ijk}	2.75^{j}
Debeira flour+10% F.M.B	104.8^{ij}	353.3^{d}	3.37^{d}
Debeira flour+15% F.M.B	108.1^{bcd}	341.7^{de}	3.16^{ef}
Debeira flour+20% F.M.B	107.7^{def}	265.0^{l}	2.46^{kl}
Debeira flour+10% F.F.B	109.6^{a}	350.0^{de}	3.19^{ef}
Debeira flour+15% F.F.B	107.7^{def}	321.7^{fg}	2.99^{gh}
Debeira flour+20%F.F.B	107.2^{def}	301.7^{hijk}	2.82^{ij}

*Any mean values in the same column having different superscript letters differ significantly ($P \leq 0.05$). F.C.B = fermented coarse wheat bran. F.M.B = fermented medium wheat bran. F.F.B = fermented fine wheat bran.

Table 4: Sensory evaluation of the bread made with different ratios of fermented wheat bran

Type of bread	Colour	Odour	Taste	Crumb texture	Crumb grain	Pref-erence
Sasaraib bread	9.3^{a}	7.5^{c}	7.5^{c}	9.1^{a}	9.3^{a}	8.7^{b}
El-Nielain bread	9.3^{a}	8.8^{a}	9.0^{a}	8.5^{c}	7.5^{c}	8.8^{b}
Debeira bread	9.3^{a}	8.9^{a}	9.0^{a}	8.9^{b}	8.3^{b}	9.1^{a}
SWF+10%F.C.B	8.2^{c}	6.8^{d}	7.0^{e}	6.6^{e}	6.7^{e}	8.0^{c}
EWF+10%F.C.B	8.8^{b}	7.3^{c}	7.8^{b}	6.5^{f}	7.3^{d}	7.8^{d}
DWF+10%F.C.B	9.3^{a}	8.0^{b}	7.4^{c}	7.3^{d}	6.8^{e}	8.3^{c}
SWF+10%F.M.B	7.1^{d}	6.7^{d}	6.5^{f}	6.5^{f}	6.3^{f}	7.0^{e}
EWF+10%F.M.B	6.0^{f}	6.5^{e}	5.3^{j}	4.7^{k}	5.2^{i}	6.5^{f}
DWF+10%F.M.B	6.9^{d}	6.7^{d}	6.4^{f}	5.9^{g}	5.8^{h}	6.9^{e}
SWF+10%F.F.B	7.0^{d}	5.4^{h}	5.5^{i}	5.7^{h}	5.7^{h}	5.3^{j}
EWF+10%F.F.B	6.5^{e}	5.7^{g}	6.2^{g}	6.7^{e}	5.7^{h}	6.3^{g}
DWF+10%F.F.B	5.9^{f}	6.1^{f}	6.1^{g}	6.4^{f}	6.5^{f}	6.6^{f}
SWF+15%F.C.B	5.1^{g}	5.9^{f}	5.2^{j}	4.7^{k}	5.1^{i}	5.9^{h}
EWF+15%F.C.B	7.1^{d}	6.4^{e}	7.3^{d}	5.7^{h}	6.1^{g}	7.5^{d}
DWF+15%F.C.B	6.9^{d}	6.0^{f}	6.0^{h}	5.6^{h}	5.7^{h}	6.3^{g}
SWF+15%F.M.B	4.0^{j}	4.9^{j}	4.6^{l}	4.4^{l}	4.4^{k}	4.4^{k}
EWF+15%F.M.B	3.7^{k}	4.7^{k}	4.1^{n}	5.5^{i}	4.6^{k}	4.3^{k}
DWF+15%F.M.B	3.5^{l}	4.3^{l}	4.7^{l}	4.8^{k}	4.7^{j}	4.2^{k}
SWF+15%F.F.B	4.2^{i}	4.8^{j}	5.1^{j}	5.2^{i}	4.5^{k}	3.9^{l}
EWF+15%F.F.B	4.4^{h}	5.3^{h}	4.4^{m}	5.3^{j}	4.6^{k}	3.8^{l}
DWF+15%F.F.B	4.4^{h}	4.7^{k}	4.9^{k}	5.5^{i}	5.1^{i}	4.7^{j}
SWF+20%F.C.B	4.7^{h}	5.3^{i}	5.9^{h}	4.2^{m}	4.9^{j}	5.1^{i}
EWF+20%F.C.B	4.3^{i}	4.5^{l}	4.7^{l}	4.7^{k}	4.7^{j}	4.3^{k}
DWF+20%F.C.B	4.3^{i}	4.6^{k}	4.5^{m}	4.5^{l}	4.2^{l}	4.2^{k}
SWF+20%F.M.B	2.1^{o}	3.3^{n}	3.3^{n}	3.2^{p}	3.6^{m}	3.0^{m}
EWF+20%F.M.B	2.4^{n}	2.5^{q}	3.1^{o}	3.2^{p}	5.0^{i}	2.5^{o}
DWF+20%F.M.B	2.0^{o}	2.9^{p}	3.0^{o}	2.6^{q}	3.1^{n}	2.6^{n}
SWF+20%F.F.B	2.6^{m}	3.3^{n}	2.7^{p}	3.5^{o}	3.5^{m}	2.8^{n}
EWF+20%F.F.B	1.4^{p}	3.1^{o}	2.5^{q}	3.7^{n}	4.1^{l}	2.5^{o}
DWF+20%F.F.B	2.7^{m}	3.6^{m}	4.4^{m}	5.4^{i}	4.7^{j}	3.6^{l}

Where: 10-9 as excellent, >9-7 as very good, >7-5 as good, >5-3 as fair, >3-1 as poor. *Any mean values in the same column having different superscript letters differ significantly ($P \leq 0.05$). EWF = El-Nielain wheat flour; SWF = Sasaraib wheat flour; DWF = Debeira wheat flour. F.C.B = fermented coarse wheat bran; F.M.B = fermented medium wheat bran; F.F.B = fermented fine wheat bran.

reported by El Mubarak *et al.* (1988) and Babiker *et al.*(1993). Tannins content of the fine wheat bran was significantly ($P \leq 0.05$) higher than that of both coarse and medium wheat bran. This difference is may be due to the fact that the fine bran is mostly seed coat.

Phytic acid content of wheat bran is shown in Table 2. Phytic acid content of non-fermented fine wheat bran was significantly ($P \leq 0.05$) higher than in non-fermented coarse and medium wheat brans. Phytic acid content of wheat bran is within the range reported by Mustafa *et al.* (2002); Elhag (1993) and Sid Ahmed (2003). But the phytic acid content of fermented wheat bran is higher than the values reported by Mustafa *et al.* (2002). This indicates that fermentation hydrolyzes the phytate by the enzyme phytase produced by the yeast, releasing the mineral elements. The hydrolyses of the phytate also improves the protein digestibility. Fig. 1 shows some minerals content in the wheat bran. Non-fermented wheat brans showed similar values of Ca and P contents. Non-fermented medium wheat bran has a higher value of Fe content compared with coarse and fine wheat bran. From the results shown in Fig. 1, it could be observed that fermentation reduced phosphorous content and increased Ca and Fe content. These results are in agreement with the results reported by Rendleman (1982) and Pomeranz and Gain (1983). Internet Report (2004) showed that wheat bran contains about 1013 mg/100 g phosphorous. Fermentation of wheat bran helps the alpha-amylase activity by releasing the Ca ion chelated with the phytic acid. The Ca ion is said to be important for the activity of alpha-amylase.

Table 3 shows the bread specific volume of wheat bread from Debeira, El-Nielain and Sasarib flours supplemented with various levels of fermented wheat bran.

Loaf specific volume decreased with the increase in the level of replacement from 4.11, 3.31 and 4.06 in the control wheat bread to 2.83, 2.11 and 2.46 in bread supplemented with 20% fermented fine bran, 20% fermented medium bran and 20% fermented coarse bran, respectively (Table 3). This drastic reduction of the bread specific volume is mainly due to the dilution of the gluten content. This deleterious effect of the bran could be reduced by using more shortening and SSL (sodium stearoyl-2-lactylate) (Mustafa *et al.*, 2002).

The sensory evaluation results (Table 4) of the bread showed the high acceptability to the bread containing

10% fermented coarse wheat bran, while the high bran breads (15, 20%) showed low acceptability, but the odour of fermented coarse wheat bran bread was liked by the panelists due to its nice rye bread like flavor. The crumb color darkens with the increase of the bran ratio and the crumb texture becomes hard. These results are comparable with the data reported by Mustafa *et al.* (2002) and Sosulski and Wu (1988).

Conclusions:

1. The four hours fermentation process decreased tannins and phytic acid contents in wheat bran.
2. Fermentation of wheat bran increases the percentage of crude fibre, protein and total carbohydrates.
3. Fermentation increases Ca and Fe in the fine wheat bran.
4. Fermented coarse wheat bran can be used to produce highly acceptable bread supplemented with up to 10% fermented wheat bran.
5. The incorporation of fermented wheat bran in bread formulation reduced the bread specific volume slightly at 10% level but significantly at the higher levels (20%) so, the addition of fermented wheat bran affects the physical and sensory properties of the baked bread.

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Comparative Survival and Growth Rate of *Clarias gariepinus* and *Heteroclarias* Hathclings Fed Live and Frozen Daphnia

R.O. Ojutiku
Department of Water Resources, Aquaculture and Fisheries,
Federal University of Technology, Minna, Niger State, Nigeria

Abstract: Feeding trial was conducted to assess survival and growth rate of *Clarias gariepinus* and *Heteroclarias* (hybrid of *Clarias gariepinus* and *Heterobranchus longifilis*) larvae (3-day old) fed on live and frozen Daphnids. Live and Frozen daphnia were used as starter diet at 50 Daphnids per larvae per feeding time for each of the species for fourteen days to assess their performance. The response to the feed and the species were compared. *Heteroclarias* and *Clarias gariepinus* fed live Daphnids performed better in terms of growth rate than those fed Frozen daphnids, though, no significant difference ($p<0.05$) statistically. *Clarias gariepinus* and *Heteroclarias* fed Frozen had greater survival than those fed live Daphnids. *Heteroclarias* fed live and Frozen Daphnids performed better in growth and survival than *Clarias gariepinus*. Therefore, Live Daphnids is recommended for larvae though Frozen Daphnids can be used as supplement and *Heteroclarias* is recommended for aqua culturists for better growth and survival.

Key words: Daphnia, *Heteroclarias*, hybrid

Introduction

Aquaculture in Nigeria is in the developing stage, because it has not been able to meet the demand and supply of the ever-increasing population. It is acknowledge as the efficient means of providing food which is rich in protein source, income and employment opportunities for the populace. Madu *et al.* (1988) noted that interest in fish culture is growing very rapidly in Nigeria but the scarcity of fingerlings of widely acceptable species of catfish such as Heterobranchus longifilis (Val. 1840) and Clarias species tend to constitute a major constraint to the rapid development of fish farming in Nigeria. Brain and Army (1980) mentioned that economically productive aquaculture like agriculture, is heavily dependent on adequate supply of seeds or fertile eggs and juvenile fish, with which to stock the pond enclosures and other culture systems. Fish culture today is hardly imaginable without the artificial or semi-artificial mass propagation of fish seeds of culture fish species. Heterobranchus and Clarias happen to be among the more than 300 species of fin fishes that have been cultivated but not spawn in captivity as reported by Brain and Army (1980) which therefore implies that their seed have to be obtained from the wild. But it was reported by Afinowi and Marioghae (1986) that supply of fingerlings from the wild as most unreliable, unstable and inadequate, since the breeding habit of most culturable species is seasoned and the fish has to be captured at the time which may not correspond to the optimum production conditions. Hybridization is practiced to achieve either of the favourable outcomes; these are heterotis or hybrid vigour, which is defined in a broad sense as increased performance value of progeny above the average of the parental performance of value. Food plays an important role in fry rearing, without which the fry cannot survive. Due to the exorbitant rate of Artemia which is a live food, there is the urgent need to source for live feed locally.

The study was therefore to determine and compare the survival and growth rate of hybrid hatchlings of Heterobranchus longifilis and *Clarias gariepinus* fed with live and frozen zooplankton (Daphina) for two weeks with the parental performance (*Clarias gariepinus*).

Materials and Methods

The brooders used for the experiment were obtained from a private farm at Ilorin and Minna Fish market, Nigeria. They were kept separately in 150cm×150cm outdoor concrete tanks at the farm and were fed maintenance ration containing 35% crude protein feed formulated before use at the farm. Brooders selections for this study were based on both external morphology and eggs characteristics. Females were selected displayed based on a rounded soft abdomen with prominent blood vessels, swollen reddish vent and appearance of viable eggs upon slight pressure on the abdomen. Using a scoop net, the brooders were removed from the concrete tanks which were further separated into species and sexes. Male, *Heterobranchus longifills*, male *Clarias gariepinus* and female, *Clarias gariepinus* were put into plastic bowls containing clean water and covered until required.

Hormone administration: Synthetic hormone, ova-prim was used in injecting the female, *Clarias gariepinus*. When administering the ova-prim, a 21-gauge needle

Table 1a: Mean length (cm) and weight (g) gain of *Clarias gariepinus* and *Heteroclarias* fed live and Frozen daphnids

	Length (cm)		Weight (g)	
	Live	Frozen	Live	Frozen
Clarias gariepinus	0.95	0.79	0.014	0.013
Heteroclarias	1.14	1.13	0.028	0.016

Table 1b: The water quality parameters monitored during the course of the experiment

	Live	Frozen
pH	7.35	7.39
Temperature (°C)	26.5	26.6
Conductivity (Us/cm)	18.15	18.15

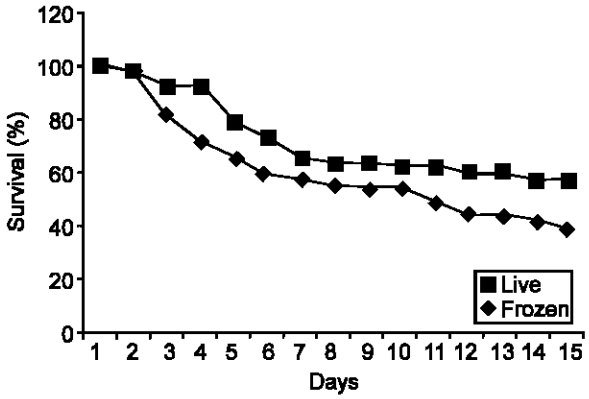

Fig. 1: Survival of *Heteroclaria* fed live and Frozen zooplankton (Daphnia)

(2.5cm) was fitted on a 5 mL syringe and the required dosage was drawn (0.5 mL of ova prim/kg of fish). The female, *Clarias gariepinus* was removed from the basin with the head covered with a head covered with a hand towel to restrain it. The syringe was aspirated to eliminate air bubbles in the hormone before the needle was inserted into the fish's body at an angle 45° in the dorsal muscle in the direction of the tail above the lateral line and retracting the syringe gently. The injected area was massaged with the thumb finger to distribute the hormone evenly. After the injection the brooders were returned into tanks containing clean water of 27°C for ovulation and maturation of gonad.

Milt collection and stripping of female fish: Before the female spawner of *Clarias gariepinus* was stripped, milt was collected from the male *Heterobranchus longifilis* and male *Clarias gariepinus* by sacrificing them. A small incision was made at the posterior end of the abdominal region of the male and the testes were removed. Testes were cleaned with tissues paper to remove blood and then kept inside a Petri dish and covered with another Petri dish until required. The female, *Clarias gariepinus* were removed from the tank check for free flowing eggs. This was done by holding firmly on the fish with a hand towel covering the head and gentle strokes were applied on the abdomen of the fish from the anterior towards the genital papilla. The eggs were stripped in to two different clean and dry plastic bowls.

Fertilization of eggs: The milt collected from the two different species were diluted with physiological saline (0.9%NaCl) solution in a ratio of 1:5 and that of *Heterobranchus longifilis* was used in fertilizing the eggs in the first bowl and that of *Clarias gariepinus* was used in fertilizing the eggs in the second bowl using a clean dry feather to avoid contamination of eggs. Fertilized eggs were incubated at a temperature range 27°C-30°C with a PH of 7.1. Three days after yolk absorption, near uniform sizes of the post larvae of *Heteroclarias* hybrid and pure *Clarias gariepinus* had their initial mean lengths and weights taken.

The experiment was set up in the indoor hatchery. Two dietary treatments (live and frozen daphnia) was used with each treatment triplicated and each of the twelve aquaria tanks containing well aerated clean water received hundred fry. Fry were fed thrice daily (0.8hrs, 13.00hrs and 18.00hrs) at an approximate rate of 50 live and 50 frozen daphnids per fry per feeding time. Water renewal was done regularly to remove uneaten foods and other metabolite to prevent fouling while about 25% of the culture medium was always replaced every morning. Such practice has been found to eliminate shock and enhance survival of cultural organisms (Peter, 1987). The survival values of the fry were determined everyday by counting and recording the mortality. The final mean weight and mean length were taken after the experiment (two weeks) to determine the growth rate.

The results were compared by using analysis of T-tests.

Results and Discussion

Result showed that the survival of *Clarias gariepinus* fed live and frozen daphnia was 15% and 26.33% respectively and the survival of the hybrid between Heterobranchus longifilis and *Clarias gariepinus* (*Heteroclarias*) fed live and frozen daphnia were 37.7% and 57% respectively (Fig. 1).

Results of Fig. 1 (treatment 1) and (treatment 2) showed that the mean survival percentage of *Heteroclarias* fed frozen Daphnia for fourteen days is higher than *Heteroclarias* fed live Daphid but the analysis did not show any significant different ($p>0.05$) in the survival.

The hybrid (*Heteroclarias*) fed frozen daphnia (57%) performed better than the *Clarias gariepinus* fed frozen daphnia (26%) and T-test showed significant difference ($p<0.05$). Also *Heteroclarias* fed live daphnia had 37% survival and performed better than *Clarias gariepinus* fed live daphnia which had 15% survival.

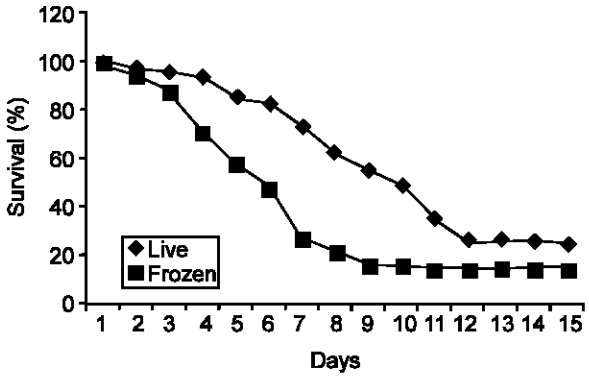

Fig. 2: Survival of *Clarias gariepinus* fed live and frozen zooplankton (daphnid)

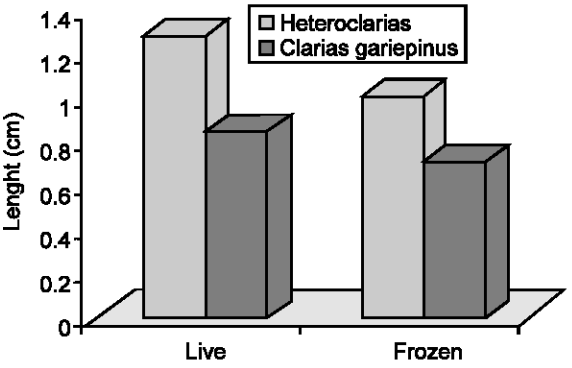

Fig. 3: Mean final length (cm) of *Clarias gariepinus* and *Heteroclaria* fed live and frozen zooplankton (daphnid)

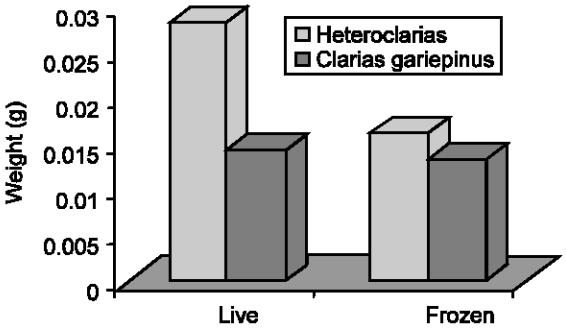

Fig. 4: Mean weight gain (g) *Heteroclaria* and *Clarias gariepinus* fed live and frozen zooplankton (Daphnia)

From Fig. 2, Table 1, the mean final length of the *Heteroclarias* fry fed live Daphnia was 1.44cm, while the mean final length of *Heteroclarias* fry fed frozen daphnia was 1.13cm though T. test analysis did not show significant difference ($p>0.05$). The mean final length of *Clarias gariepinus* fry fed live Daphnia was 0.95cm and mean final length of *Clarias gariepinus* fed frozen daphnia was 0.79cm. The mean final weight gain of *Heteroclarias* fed live and frozen daphnia are 0.028gm and 0.016gm respectively while the mean weight gain of *Clarias gariepinus* fry fed live and frozen daphnia are 0.0135gm and 0.0126gm respectively (Table 1, Fig. 2) which agrees with the findings of Ovie and Adepoju (1995) and Lamai (1999) who reported that larva fed live daphnia gave the best performance in terms of growth. *Hereroclarias* fed live daphnia performed better in terms of weight gain than the *Clarias gariepinus* fed live which is the pure breed. This implies that Hybrid performs much better than either of the parents because of the improved hybrid vigour. This agrees with the Olarewaju and Dada (1997) who observed that hybrid in most cases were superior to the parent strains in growth; food conversion and resistance to diseases. The development of simple methods and techniques for indigenous zooplankton production would, not only improve the hatchery management of cat fish but would also reduce the cost of production and improve profit margin of hatchery operation because Artemia is expensive because of import tarrif.

In conclusion, *Heteroclarias* should be encouraged because it performed better and indigenous zooplankton should be promoted because it will drastically reduce the cost of production.

From the table above, it is clear that the water parameters were within tolerable limits for fish culture.

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Effect of Different Cereals on the Quality of *Masa*

J.A. Ayo, H. Agu and O.F. Famoriyo
Department of Food Science and Technology, The Federal Polytechnic, Bauchi, Nigeria

Abstract: The pearl millet and maize grains were dehulled, washed, dried and ground while the rice grain was ground using disc attrition grinder. The powdered grain is sieved to produce flour and grit which is added to boiling water and cooked before mixing with the raw flour in the of 1:2. The resulting batter is inoculated with baker's yeast and allowed to ferment (14-16hrs), diluted with trona (kanwa water), salted, stirred vigorously to incorporate air, sized, fried in little oil (3mins on one side, then turned) to produce *masa*. The *masa* (rice, maze and millet based) were analysed for physical (thickness, volume, spread ratio), chemical (fat, moisture, ash, protein, carbohydrate) and sensory (colour, texture, taste, odour) qualities. The dimension of *masa* ranged from 8.40 to 8.97cm in diameter, 2.70 to 3.10cm thickness, 79 to 88.9g weight, 115.57 to130.35cm^3 loaf volume and 1.30 to 1.67 loaf volume index. The proximate composition of the *masa* samples range from 7.59 to 9.21% for protein. 8.82 to 9.60% for fat, 1.8 to 2.1% ash, and 75.16 to 76.99% carbohydrate. The sensory quality means scores range from 5.27 to 8.4 for taste, 6.67 to 8.82 for texture, 5.60 to 7.87 for odour, 2.53 to 8.80 for colour, 6.27 to 8.67 for appearance and 5.27 to 8.67 for general acceptability. Generally maize based *masa* compare favourably with rice based which has been the commonly used cereal for the production.

Key words: *Masa*, cereals, pearl millet, maize grains

Introduction

Masa (waina) is a fermented puff batter or bread like of rice or maize cooked in a pan with individual cuplike depression. *Masa* (or waina) is like the India idle in shape and *dosa* in taste (Nkama *et al*., 1998) and different from the Mexican '*Masa*' used in tortilla preparation. *Masa* is a very popular staple food consumed by over 80% of the Northern Nigeria population of about 47 million (Nkama, 1993). It is also consumed in Niger, Burkina Faso and Mali (Nkama, 1998). *Masa* is prepared to create variety in cereal for sale; it serves as breakfast and snack item. Though m*asa* is as popular as Nigeria *Ogi,* it receives very little attraction (Nkama and Malleshi, 1998).

A fairly large numbers of research works has been carried out on cereal products (Bacon, 1980; Badi *et al*., 1990; Banigo, 1997; Chavon and Kadam, 1997; Desikachar, 1975; Hofvanda and Underwood, 1997; Hubbel *et al*., 1997; Khetarpaul and Chauhan, 1991) but not much on *masa*.

M*asa* is consumed in various forms by all aged groups in the Northern states of Nigeria. *Masa* which results from frying of the fermented dough which is round in shape with brown smooth boy and crippling edges. The brown crisp edges and the mild sour taste are considered by many consumers as the quality attribute required of m*asa*.

Masa is a good source of income for the waina who prepares the traditional product on sale. The addition of cowpea, groundnut or soybeans flour into m*asa* during preparation improved the nutritional quality of m*asa* (Nkama and Malleshi, 1998). It serves as a breakfast and snack item. Though m*asa* is as popular as Nigeria ogi, it has received very little attention (Nkama and Muller 1989).

The raw materials and ingredient including millet, rice, salt, sugar, yeast, *trona* or *mkanwa*, vegetable oil are used. The grain particularly pearl millet or maize is dehulled (rice and acha are used directly), washed, soaked (12hrs), dried and milled (disc attrition mill). The ground rice/maze/millet is sieved to produce flour and grits. The grits are added to boiling water and cooked to gelatinization and allowed to cool before mixing with raw flour in the ratio of 1:4. The resulting batter inoculated with bakers yeast and its allowed to ferment over night (14-16hours), salt and sugar are added to the inoculums. The fairly thick batter is then diluted with *trona* (*Kanwa* water) an the batter is stirred (vigorously to incorporate air) and fried in a cup-like depression in which oil has been added to produce *masa*.

The problem of *masa* apart from the short shelf keeping quality, is that inconsistence in the use of varied cereals and spices which has resulted in variations in the quality of the product.

The aim of the work is to asses the effect of different types of cereals on the quality of *masa*.

Materials and Methods

Raw rice (*Oryza Sativa* L.), pearl millet (Pennisetum americanum), maize (*Zea mays* L.) and active bakers yeast (*Saccaronyces cerevesiae*) used for the work were purchased bulk from Jos Central Market, Plateau State. *Kanwa* or *trona* (Sodium bicarbonate) was purchased

Table 1:Reciepe for *masa* production

Raw Materials (g)	Samples A	B	C
Rice(g)	500	-	-
Millet(g)	-	500	-
Maize(g)	-	-	500
Water(cm)	600	600	600
Sugar(g)	30	30	30
*Trona(cm)	10	10	10
Yeast(g)	5	5	5
Frying oil(cm)	12	12	12
Salt	Pinch	Pinch	Pinch

* 20% solution of Trona.

from Yelwa Market, Bauchi State, Nigeria. The recipe for production of *masa* is shown in Table 1. Raw milled rice was cleaned, washed, soaked (for 12hours at 34°C),¼ of the rice was cooked and mixed with the ¾ portion (milled into powder). The resulting batter was inoculated with bakers yeast (1.0%) and allowed to ferment overnight (14-16hours at room temperature 38°C). The fairly thick batter was then diluted with 10cm^3 *trona solution* (20%). Salt (pitch) and sugar (6%) was added to the batter, stirred vigorously (using a mortar and pestle to incorporate air) and fried (in a local clay pot with individual cuplike depression in which 12cm^3 oil has been added). The batter was fried for 4 minutes on one side, then turned with a small spoon and the other side fried (frying time varies from 6 to 8 minutes) to produce *masa*.

The thickness and width of the *masa* ball was measured using micrometer and ruler, respectively. The loaf volume was determined using seed-displacement method (Ayo, 2003), while the loaf volume was calculated by dividing the loaf volume by the weight of the *masa* (Gomez *et al.*, 1997). The chemical quality (moisture, fat, protein, ash and carbohydrate) were determined (AOAC, 1990). The sensory qualities of the *masa* were later subjected to sensory evaluation by 20 untrained panellists (students and staff) from the polytechnic community. Attributes assessed include flavour, taste, colour, texture appearance and the overall acceptability of m*asa* using Nine Hedonic scale (1 and 9 for extremely dislike and extremely like, respectively). The data collected were analysed using ANOVA method (Ihekoronye and Ngoddy, 1985).

Results and Discussion

Effect of different cereals on the physical quality of *masa:* The effect of different type of cereal grain (rice, maize and millet) on the physical quality of *masa* is summarized in Table 2. The average thickness and length of the rice, maize and millet grains based *masa* were 3.10 and 8.53, 3.43 and 8.97 and 2.70cm and 8.40, respectively. The average loaf volumes of the rice, maize and millet bases *masa* were 130.4, 129.5 and 115.6cm^3, with a corresponding index of 1.65, 1.59 and 1.30,

Table 2: Effects of different cereals on the physical qualities of *masa*

Parameters	Rice	Maize	Millet
Thickness (cm)	3.10±0.3^a	3.43±0.6^a	2.70±0.7^b
Length (cm)	8.53±0.2^b	8.97±0.2^a	8.40±1.2^b
Weight (g)	79.0±9.2^b	81.4±0.4^b	88.9±6.3^a
Loaf volume (cm)	130.4±5.6^a	129.5±0.3^a	115.6±8.2^b
Loaf volume index(cm^3/g)	1.65±0.2^a	1.59±0.2^a	1.30±0.4^b

respectively. There were no significant difference in the length, volume and volume index of rice and maize based *masa*. The none significance differences, p = 0.05, could be due to similarity in the molecular weight and structures of carbohydrates which are the principal functions of volume development during fermentation (Chavon and Kadam, 1997).

Effect of different cereals on the chemical quality of *masa*: The effect of different types of cereal grains (rice, maize and millet) on the chemical quality of *masa* are summarized in Table 3. The average protein content of m*asa* produced from rice, maize and millet are 8.59, 9.60 and 9.21%, respectively. The relative difference could be due to the chemical composition of the raw materials (cereal). Protein is found in all tissue of cereal grains but the concentration varies from grain to grain (Kent 1984). Rice grain has protein content of 6.8-8.0% while maize and millet have protein content of 9-10% (Kent, 1984; Ihekoronye and Ngoddy, 1985) which is in agreement with the observations.

The average ash (mineral) and fat content of *masa* produced from rice, maize and millet were 1.8 and 9.82, 2.0 and 9.47 and 2.1 and 9.60% respectively. The relatively higher ash content in the respective *masa* could be due to the addition of trona and salt added during production. The relatively high fat content despite the low fat/ oil level of the raw material (cereal) could be due the oil used in toasting with its tendency of been absorbed by the batter. The relatively high oil content in *masa* could endanger the keeping quality of the product, which could be related to the short shelf life of the products as observed by Nkama (1993,1998).

The average moisture contents were 14.80, 13.81 and 12.4% for rice, maize and millet based *masa*, respectively. The cereal based *masa* are relatively high in moisture content which could encourage growth of microbes (Okaka, 2005) within short time. The carbohydrate contents were 64.99, 65.16 and 66.66% for rice, maize and millet based *masa*, respectively. There is no significant deference between the carbohydrates of the cereal based *masa*, p=0.05. The relatively high carbohydrate content could make the product of significant source of energy to the consumers.

The effect of different cereal on the sensory quality of *masa*: The effects of rice maize and millet in the sensory quality of *masa* are summarized in Table 4. The average means score for taste of *masa* produced from rice,

Table 3: Effect of different cereal on the chemical qualities of *masa*

Samplesss Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	CHO (%)	Calorie Kcal/100g
Rice	14.8±0.7	7.59±0.5[b]	9.82±.8[b]	1.8±01[b]	66.99±5.3[a]	387.70[a]
Maize	13.81±0.4	9.56±0.4[a]	9.47±0.4[a]	2.0±0.3[a]	65.16±6.1[a]	384.11[a]
Millet	12.43±1.2	9.21±0.7[a]	9.60±0.6[a]	2.1±0.2[a]	66.66±6.4[a]	412.28[a]

Table 4: Effect of different cereals on the sensory qualities of *masa*

Raw Materials	Taste	Texture	Odour	Colour	Appearance
Rice	8.40±.507[a]	8.02±.676[a]	7.87±.640[a]	8.80±.414[a]	8.67±.488[a]
Maize	8.07±.704[a]	7.87±.95[a]	6.80±.561[b]	8.40±.507[a]	8.40±.507[a]
Millet	5.27±.799[b]	6.67±.90[b]	5.60±1.056[c]	2.53±1.302[b]	6.27±.961[b]

Mean score having the same alphabet along the same column are not significantly different p = .05

maize and millet were 8.40, 8.7 and 5.70 respectively. There was no significant difference (p = 0.05) between rice and maize in terms of taste. Rice had the highest (8.40) means score for taste and the reason could be because rice is commonly used for the production of *masa* millet was poorly accepted.

The average means score for texture of *masa* produced from rice, maize and millet were 8.20, 7.87 and 6.67 respectively. Low fibre content of flours generally has been observed to improve baking quality of the baked products which could be the reason for the rice based *masa* with 0.7% fibre content. Maze and millet with relatively higher fibre content of 1.2 and 3.0% respectively. Kordylas (1990) have been found to have poor texture quality.

The average mean score for odor of *masa* from rice, maize and millet were 7.87, 6.80 and 5.60 respectively. There were significant different between this cereal based grains with rice having the highest (7.87) and this reason could be due to the adaptability of the consumers to the rice based *masa*

The average mean score for colour of *masa* produce from rice, maize and millet were 8.80, 8.40 and 2.53 respectively. There were no significant difference (p = 0.05) between rice and maize in terms of colour. The significant differences of millet based *masa* could be due to the presence of colouring pigment which is inherent in millet flour.

The average mean score for appearance of *masa* produced were rice (8.67), maize (8.40) and millet (6.27). There were no significant difference between rice and maize the reason could be that *masa* produced from rice and maize are alike in appearance while millet looks different (in term of colour).

There was significant difference for general acceptability. The coverage men score for rice; maize and millet were 8.67, 8.13 and 5.27 respectively. Maize was the next cereal grain accepted while millet was poorly accepted.

Conclusion

Rice, maize and millet can be used to produce *masa*. However, there is no significant difference in both the physical, chemical and sensory quality of rice and maize based *masa* but there is slight difference with that of millet. It can therefore be said that maize could substitute the relatively costly and highly demanded rice in the production of *masa*. The adoption of maize in the production of *masa* will fairly increase the protein content from 7.59 to 9.56% (an increase of 1.97%). Because of the relatively cheap price of maize (1kg of maize is N50.00 and rice of the same quantity is N150.00), maize based *masa* could be cheaper and affordable by the masses.

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Effect of Exogenous Enzymes on the Growing Performance of Broiler Chickens Fed Regular Corn/Soybean-Based Diets and the Economics of Enzyme Supplementation

Hana A.H. Zakaria[1], Mohammad A.R. Jalal[1] and Amer S. Jabarin[2]
[1]Deaprtment of Animal Production, Faculty of Agriculture, The University of Jordan, Amman, Jordan
[2]Department of Agricultural Economics and Agribusiness, The University of Jordan, Amman, Jordan

Abstract: A study was conducted to compare the effects of two sources of exogenous enzymes on performance of broilers fed regular corn/soybean diets and economics of enzyme supplementation. Bergazym P (Berg) and Hemicell-D (Hemi) were added at rates of 0.025 and 0.05%, respectively, to a control diet (Con) for starter (1-21 d) and finisher (22-42) phases in a completely randomized design. Birds were randomly allotted according to body weight to 3 dietary treatments with 12 replicate per treatment (50 chicks per replicate). Enzyme supplementation had no significant effect of feed intake (FI) at 21 and/or 42 days, even though, enzyme-supplemented birds consumed more feed than Con. Birds fed Berg and Con diets had comparable feed conversion ratios (FCR) at 42 days and were significantly ($P<0.05$) more efficient than Hemi diet. Body weight (BW) was not significantly affected by enzyme supplementation, however, birds fed Con and Berg weighed heavier than Hemi group at 42 days. Body weight gain (BWG) was numerically greater for birds fed Con and Berg diets at 21 and/or 42 days compared to Hemi diet, despite no significance reported. Economic analysis showed no monetary benefits from including the enzymes in poultry diets as there were no significant differences in final BW among the three diets. Furthermore, birds fed Con diets weighed heavier than birds fed enzyme-supplemented diets. Calculations for the three budgets show a reduction in gross margins and cost-benefit ratio when Berg and Hemi were added compared to Con diet due to reductions in final BW.

Key words: Enzyme, broiler chicken, performance, corn-soybean diets, economic analysis

Introduction

Feed ingredients of plant origin contain a number of components that are refractive to monogastric digestive enzymes because of lack of and/or insufficiency of endogenous enzyme secretions (Ravindran *et al*., 1999). These components also lower the utilization of other dietary nutrients, leading to a reduced bird performance. Examples of such antinutritive components include β-glucans in barley, pentosans in wheat, and certain oligosaccharides in soybean meal (Annison and Choct, 1991). Therefore, development of commercially-available feed enzymes to target specific substrates and ameliorate antinutritive effects has received increased attention in the last decade.

The use of exogenous substrate specific enzymes (xylanase, beta-glucanase, amylase, etc...) in poultry feeds to improve bird performance is not a new concept and had been extensively researched (Bedford and Schulze, 1998; Bedford, 2000; Acamovic, 2001; Cowieson and Adeola, 2005). These improvements are related to greater digestion and absorption of nutrients in cereal grains caused by the degradation of cell wall nonstarch polysaccharides (NSP) (Bedford and Schulz, 1998). The degradation of NSP has been proposed as the underlying mechanism to improve bird performance by releasing nutrients trapped within the cell and lowering digesta viscosity to enhance nutrient digestion and subsequent absorption (Classen and Bedford, 1991; Bedford and Schulze, 1998).

Therefore the purpose of this study was to determine the effects of two sources of exogenous enzymes on production parameters in straight run broiler chickens fed corn-soybean based diets and evaluate the economics of their supplementation.

Materials and Methods

Animal trial: A total of 900 straight run Hubbard broilers obtained from a local commercial hatchery were used in a growth performance trial. Birds were randomly allotted according to body weight uniformity to three dietary treatments with 12 replicate floor pens of 50 chicks per replicate (300 chicks per treatment). A regular corn/soybean-based diet was formulated and used as a control diet without enzyme for both starter (0-21 days) and finisher phases (22-42 days) (Table 1). The control diet (Con) was supplemented separately with the commercial enzymes Bergazym P® (Berg) and Hemicell® (Hemi) to make up diets 2 and 3, respectively, for both

starter and finisher phases. Bergazym P® is a non-genetically modified multi-microbial enzyme (Berg and Schmidt[1]) produced by *Trichoderma longibrachiatum* containing a leading activity of 6,000 units of endo-1,4-β-xylanase/g (activity determined by manufacturer) with adjusted side activities (β - glucanase, amylases and galactomannase). Hemicell-D® is a non-genetically modified enzyme (Chem Gen Corporation[2]) produced by *Bacillus lentus* containing a minimum of activity of 14,000 of 1,4-β-mannanase/g. Berg and Hemi were added to Con diet at rates of 0.025% (substitute 50 ME kcal/kg feed) and 0.05% (substitute 100 kcal ME/kg) of the diet, respectively.

Chicks were reared from 1 d old on the experimental diets and were allowed ad libitum access to feed and water throughout the study. All diets were fed in mesh form. Pens had a daily lighting regimen of 20 h of light and 4 h of dark; room temperature was maintained at 35°C in the first week and was reduced by 2°C every week until maintained at 25°C. Birds were reared in an open-sided house on floor pens. All animals used in this trial were handled in accordance with guidelines set forth by the Jordanian Society for the Protection of Animals.

Parameters measured on a weekly basis included feed intake (FI), feed conversion ratio (FCR), bodyweight (BW), bodyweight gain (BWG) and mortality. Experimental diets were formulated according to the breeder's manual (Hubbard S.A.S) to meet National Research Council (1994) requirements of broilers. The duration of the trial was 42 days.

Data was analyzed using the Proc Mixed procedure of SAS® for repeated measures analysis for a completely randomized design and was tested for main effect of treatment. The following general linear model was used:

$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$

Where Y_{ij}= measured response; μ = overall Mean; α_i = dietary treatment effect; ϵ_{ij}= residual error.

Data was also tested for the following contrasts:

Con vs. Enzymes (Control Diet vs. Enzyme-Supplemented Diets).

Berg vs. Hemi (Bergazym Diet vs. Hemicell Diet).

Level of significance used was p = 0.05

Economic analysis: The economic evaluation was conducted to assess the feasibility of using the two enzymes in broiler feed. Enterprise budgets are usually used in economic analysis to estimate the profitability of any agricultural activity. In this research several enterprise budgets for Hubbard broilers were constructed using day-to-day collected data. The budget shows the total revenue from selling the broiler at the local market evaluated at the farm gate price. The budgets also show the different types of costs involved in the production process. The costs are classified into variable and fixed costs. The variable costs are those cost items that change with the variation of the production level while the fixed costs do not change with the variation in production. The enterprise budget shows the costs of the different items involved in the production process for each treatment of 300 chicks.

Different economic indicators are used in this paper including: 1) gross margins per 300 chicks; 2) net returns and 3) benefit cost ratio. Gross margin per each 300 chicks is estimated by deducting the total variable costs from the total revenues. The net returns represent the net profit per 300 chicks that is estimated by deducting the total costs from total revenues. The benefit cost ratio is obtained by dividing the total revenues by the total costs per 300 chicks.

The three economic indicators were used in this paper to evaluate in economic terms the impact of using the two enzymes on the broiler production compared to traditional methods (control group).

Table 1: Diet composition

Ingredients	Starter (0-21 days)	Finisher (21-42 days)
	(%)	
Corn	53.750	62.360
Soybean meal (48.5 % CP)	39.800	30.000
Soybean oil	2.600	3.760
Limestone	0.860	0.900
Dicalcium phosphate	1.770	1.700
NaCl	0.430	0.430
DL-methionine (98%)	0.220	0.250
L-Lysine-HCl	0.220	0.250
Vitamin premix[1]	0.125	0.125
Mineral premix[2]	0.125	0.125
Choline chloride (60%)	0.100	0.100
Calculated Nutrient Composition:		
ME, kcal/kg	3,000	3,150
Protein, %	22.50	19.00
TSAA, %	0.92	0.83
Methionine, %	0.53	0.49
Lysine, %	1.38	1.28
Ca, %	0.96	0.92
P, nonphytate, %	0.45	0.43
Na, %	0.18	0.18

[1]Vitamin premix provided per kilogram of diet: vitamin A, 120000 IU; vitamin D_3, 3500 IU; vitamin E, 40 mg; vitamin B_1, 2.5 mg; vitamin B_2, 8 mg; vitamin B_6, 5.0 mg; vitamin, riboflavin, 150 µg; B_{12}, 30 µg; biotin, 150 µg; folic acid, 1.5 mg; niacin, 45 mg; pantothenic acid, 13 mg. [2]Trace mineral premix provided per kilogram of diet: Fe, 30 mg; Cu, 15 mg; Mn, 60 mg; Zn, 550 mg; I, 1 mg; Se, 0.80 mg.

Results and Discussion

Enzyme supplementation had no significant effect on feed intake in contrast to the Con at 21 and/or 42 days, even though birds fed diets supplemented with enzymes

[1]Berg and Schmidt, An der Alster 81, D-20099 Hamburg, Germany.

2ChemGen Corporation, 211 Perry Parkway, Gaithersburg, MD 20877-2144, USA.

Table 2: Production data - feed intake, feed conversion, body weight and body weight gain

Production Parameter	Feed Intake (g/bird)		Feed Conversion Ratio (g feed:g weight gain)		Body Weight (g)		Body Weight Gain (g/bird)	
Days	21	42	21	42	21	42	21	42
Diets:[1]								
1) Con	828.48	3550.72	1.57	1.82[a]	572.21	1929.03	528.21	1884.14
2) Berg[2]	840.61	3595.65	1.70	1.82[a]	539.78	1916.67	495.15	1872.96
3) Hemi[2]	854.77	3481.79	1.82	2.06[b]	517.61	1760.81	473.15	1716.34
SEM	11.77	75.07	0.127	0.077	25.01	80.70	24.73	80.31
Diet Effect	NS	NS	NS	0.03	NS	NS	NS	NS
Contrasts:								
Con vs Enzymes	NS	NS	NS	NS	NS	NS	NS	NS
Berg vs. Hemi	NS	NS	NS	0.02	NS	NS	NS	NS

[a-b] Means with varying superscripts differ significantly (P<0.05). [1]Diets: Control (Con), Bergazym P (Berg)[2], Hemicell (Hemi)[2].
[2]Berg = Bergazym P (Berg and Schmidt, An der Alster 81, D-20099 Hamburg, Germany); Hemi = Hemicell-D (ChemGen Corporation, 211 Perry Parkway, Gaithersburg, MD 20877-2144, USA).

Table 3: Input-output data and gross margin for broiler enterprise [Unit of 900 broilers per one cycle (42 days)] – Control Diet

	Unit	Quantity	Price Per unit	Value JD
Broilers: (900 - 6 (mortality)=894 sold birds@1.92kg)	Kg	1716.48	0.88	1510. 5
Manure :	JD	1	11	11. 0
A. Gross revenue				1521. 5
Variable costs				
Purchase of day old chicks:	chick	900	0.2	180. 0
Feed mixture	kg	2700	0.25	675. 0
Bed preparation	sack	27	1.55	41. 9
Gas (Liquid propone)	Jar	45	3.5	157. 5
Water	Cubic meter	15	5	75. 0
Electricity	JD	1	30	30. 0
Veterinary expenses	JD	1	33.3	33. 3
Disinfectants	JD	1	30	30. 0
Labor cost	JD	1	183	183. 0
Interest on operating capital (9% yearly, 0.75% *2 months *JD 1468 total variable costs)				21. 1
B .Total variable costs	JD			1426. 7
Fixed costs				
Rent of building	JD			60. 0
Interest on fixed capital (9% yearly, 0.75% *2 months *JD 60 total fixed costs)				0. 9
C. Total fixed costs	JD			60. 9
D. Total costs (b+c)	JD			1487. 6
E. Gross margin (a-b)	JD			94. 8
F. Net profit (a-d)	JD			33. 9

numerically consumed more grams than control (Table 2). Consistent with our results, previous research with xylanase (Ravidran *et al.*, 1999; Cafe *et al.*, 2002; Cowieson and Adeola, 2005) cited no effects on FI of broilers when supplemented to cereal-based diets (corn, wheat, or barely). Saki *et al.* (2005) and Zou *et al.* (2006) reported no significant differences in average FC at 21 and 42 days in broilers fed diets supplemented with β-mannanase at 0.05% of the diet. Both experiments were conducted with corn-soybean based diets similar to those used in our trial.

Feed conversion ratio was significantly (P<0.05) affected by enzyme supplementation at 42 days (Table 2). Bergazym-supplemented group exhibited FCR at a level comparable with Con group, and significantly lower than Hemi group. Broilers fed Hemi diet were significantly less efficient in converting feed to gain in contrast to Con and Berg groups by 0.24 units. Xylanase supplementation has been shown to produce a 2.2% reduction in FCR when supplemented in an enzyme mixture to broilers fed regular corn-soy diets (Zanella *et al.*, 1999). More recent work (Mathlouthi *et al.*, 2002; Wu and Ravindran, 2004) with other cereal grains (rye and wheat) has shown that xylanase supplementation greatly reduced feed/gain or FCR in broiler chickens. In disagreement with our findings with Hemi, Zou *et al.*

[1]Berg and Schmidt, An der Alster 81, D-20099 Hamburg, Germany.
2ChemGen Corporation, 211 Perry Parkway, Gaithersburg, MD 20877-2144, USA.

Table 4: Input-output data and gross margin for broiler enterprise [Unit of 900 broilers per one cycle (42 days)] - Hemi Diet

	Unit	Quantity	Price Per unit	Value JD
Broilers : (900 - 16 (mortality) = 884 sold birds@1.76kg)	Kg	1555.84	0.88	1369. 1
Manure :	JD	1	11	11.0
A. Gross revenue				1380.1
Variable costs				
Purchase of day old chicks:	Chick	900	0.2	180.0
Feed mixture	Kg	2700	0.25	675.0
Bed preparation	Sack	27	1.55	41.9
Gas (Liquid propane)	Jar	45	3.5	157.5
Water	Cubic meter	15	5	75.0
Electricity	JD	1	30	30.0
Veterinary expenses	JD	1	33.3	33.3
Disinfectants	JD	1	30	30.0
Labor cost	JD	1	183	183.0
Hemi enzyme	Kg	0.45	4	1.8
Interest on operating capital (9% yearly, 0.75% *2 months *JD 1407.5 total variable costs)				21.1
B. Total variable costs	JD			1428.5
Fixed costs				
Rent of building	JD			60.0
Interest on fixed capital(9% yearly, 0.75% *2 months *JD 60 total fixed costs)				0.9
C. Total fixed costs	JD			60.9
D. Total costs (b+c)	JD			1489.4
E. Gross margin (a-b)	JD			-48.4
F. Net profit (a-d)	JD			-109.3

(2006) found that Hemicell greatly improved FCR when supplemented to regular corn-soybean diets at an inclusion rate of 0.05% of the diet. Jackson *et al.* (2004) reported inclusion of β-mannanase at 80 million U/ton improved FCR at 42 days, while Daskiran *et al.* (2004) cited reduced FCR to values or better than control at 0.5, 1.0 and 1.5% inclusion rates at 42 days in broiler diets. Trials conducted with swine have shown lowered insulin secretion due to β-mannan contained in soybean meal and (Leeds *et al.*, 1980; Sambrook and Rainbird, 1985) and a reduction in glucose absorption (Rainbird *et al.*, 1984). It has been therefore, postulated that addition of Hemicell may ameliorate insulin secretion and glucose absorption by hydrolyzing β-mannan (Zou *et al.*, 2006), and improve energy metabolism. In this experiment, addition of 0.05% Hemicell failed to elicit any response even though 0.05% is within the range of the manufacturer's recommendations. There is no explanation for this finding previously reported by Saki *et al.* (2005) at similar inclusion rates.

There were no significant differences in BW 21 and/or 42 days due to enzyme supplementation (Table 2). However, weights of broilers fed Con and Berg diets were numerically greater than those in the Hemi group with a difference of 168 and 156 g/bird, respectively, in final BW. A similar trend was observed with regard to BWG, with birds in the Con and Berg groups exhibiting BWG at 21 and/or 42 days in contrast to birds supplemented with Hemi, even though differences were not significant (Table 2). Cafe *et al.* (2002) reported significant improvement in final BW at 49 days in broilers fed xylanase-supplemented diets in contrast to a control corn-based diet. Contrary to our results, response to xylanase supplementation has been reported with regard to BWG in broilers. Studies by Zanella *et al.* (1999), Mathlouthi *et al.* (2002) and Wu and Ravindran (2004) in broilers and Adeola and Bedford (2004) in ducks have all cited increased weight gains in birds fed cereal-based diets supplemented with xylanase. In this experiment, Hemi did not improve BW 21 and/or 42 days. The results are inconsistent with findings of Saki *et al.* (2005) who reported that Hemi increased 42 day BW of broilers by 60 g/bird when added at 0.05%. In contrast to our results, previous experiments (McNaughton *et al.*, 1998; Jackson *et al.*, 2004; Saki *et al.*, 2005; Zou *et al.*, 2006) all reported improved weight gains in broilers due to Hemi supplementation at 42 days. As indicated earlier, this is potentially related to an improvement in energy utilization, anticipated with reduced β-mannan intake.

In this experiment, supplementation of xylanase and β-mannanase to broiler diets only resulted in a significant effect on FCR. No significant effects and/or improvements were observed on FI, BW and BWG. The beneficial effects of xylanase are more evident in diets based on barley, wheat, and rye which contain higher NSP content than corn (Cowieson, 2005) which has less than 1 g/kg (Choct, 1997). We have indicated earlier in the discussion of improved performance in broilers fed on corn/soybean based diets when supplemented with xylanase. However, in these trials xylanase was

Table 5: Input-output data and gross margin for broiler enterprise [Unit of 900 broilers per one cycle (42 days)] - Berg Diet

	Unit	Quantity	Price Per unit	Value JD
Broilers: (900- 13 (mortality) = 887 sold birds@ 1.916 kg)	Kg	1699.492	0.88	1495. 6
Manure:	JD	1	11	11. 0
A. Gross revenue				1506. 6
Variable costs				
Purchase of day old chicks:	Chick	900	0.2	180. 0
Feed mixture	Kg	2700	0.25	675. 0
Bed preparation	Sack	27	1.55	41. 9
Gas (Liquid Propane)	Jar	45	3.5	157. 5
Water	Cubic meter	15	5	75. 0
Electricity	JD	1	30	30. 0
Veterinary expenses	JD	1	33.3	33. 3
Disinfectants	JD	1	30	30. 0
Labor cost	JD	1	183	183. 0
Berg enzyme	Kg	0.225	5	1. 1
Interest on operating capital(9% yearly , 0.75% *2 months *JD 1406.8 total variable costs)				21. 1
B. Total variable costs	JD			1427. 9
Fixed costs				
Rent of building	JD			60. 0
Interest on fixed capital (9% yearly, 0.75% *2 months *JD 60 total fixed costs)				0. 9
C. Total fixed costs	JD			60. 9
D. Total costs (b+c)	JD			1488. 8
E. Gross margin (a-b)	JD			78. 7
F. Net profit (a-d)	JD			17. 8

supplemented in combination with other exogenous enzymes such as protease, amylase or phytase in contrast to individual use which is the case in our trial. The independent use of xylanase is unlikely to yield the value or consistency of response that a multiple of enzyme activities is capable of providing (Cowieson, 2005).

Supplementation of β-mannanase did not improve performance of broilers and even the FCR was significantly lower than xylanase and control diet. It has been shown that the large effects of β-mannanase on performance cannot be explained simply in terms making β-mannan available as an energy source (Jackson *et al.*, 2004). The β-mannan content of soybean meal is only about 1.1 to 1.3% (Dierick, 1989) and in complete diets 0.4 to 0.7% (Jackson *et al.*, 2004). The mode of action of this enzyme is complex. β-mannans are highly viscous and have adverse effects on digestive system and are associated with feedstuffs such as barely, wheat and rye. Enhanced performance may occur due to use of this enzyme in combination with other enzymes in broilers fed diets based on viscous cereals (Jackson *et al.*, 2004). In our trial, β-mannanase was used individually and for broilers fed corn based diets.

As indicated above, the results show that there is no significant difference in the broilers' weights in the control group and the two groups fed diets supplemented with the two tested enzymes. On the contrary, the addition of the enzymes reduced the body weights of the broilers. This reduction in body weight in addition to the cost of adding the enzymes has resulted in economic losses to the producer. Table 3 shows the enterprise budget for producing 300 Hubard broilers under control conditions. While Tables 4 and 5 include the enterprise budgets for producing 300 broilers using diets with added Hemi and Berg enzymes, respectively. The calculations in Tables 3 through 5 demonstrate that gross margins per the 300 broilers decreased from JD 94.8 (US$ 134) (the control diet) to JD -48.4 (US$ -69) when the Hemi enzyme was added to the diet. However, when Berg enzyme was added the gross margins per the 300 broilers decreased to JD 78.7 (US$ 111) compared to the control diet.

The estimated benefit-cost ratio using the three budgets shows that at the Con diet the ratio was 1.07 while when the Hemi and Berg enzymes were added to the diets, the ratio dropped to 0.97 and 1.05, respectively.

Conclusions: It is obvious from the results of the above analysis that both Berg and Hemi when supplemented individually did not improve performance except with FCR which was similar for Berg and Con diets, whilst Hemi-supplemented birds were less efficient. It is likely that a better response could be obtained if both enzymes were supplemented together and or in combination with other enzymes such as phytases or proteases. It is also important to note that we used a corn-based diet, as corn is the predominant cereal grain imported and used by poultry producers in Jordan. Further research is

needed on utilizing these enzymes simultaneously and in combination with other exogenous enzymes and also an investigation of their effects on morphology and viscosity of the digestive tract.

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Chemical Constituents of Cowry (*Cyparica samplomoneta*)

O.I. Oloyede
Department of Biochemistry, University of Ado-Ekiti, Nigeria

Abstract: Perforated cowry shells (*Cyparica samplometa*) were tested for the presence of certain phytochemicals, minerals Proximate analysis were also determined. Cowry shells (dry weight) showed the presence of Alkaloid, Cardiac glycosides, Tannins and quinones. Chemical analysis revealed the presence of Calcium (91.35±0.45 mgl100g) and Iron (47.52±0.02 mg/100g) as well as Aluminum and Sodium in considerable quantities. Proximate analysis showed that it contained moisture content (0.22%), Ash content (76.30%), Crude fibre (7.27%), Crude protein (5.10%) carbohydrate (14.13%) and Crude fat (0.42%). The presence of some important phytochemicals and calcium in highest quantity explains the action of the cowry shells encountered in therapeutic uses and their involvement in pharmaceutical product. The extraction of bioactive agent of cowries shell is one of the most intensive area of natural product research.

Key words: Cowry shell, phytochemicals, natural product research

Introduction

Cowries generally belong to the member of mollusks and family of *Cypraeidae*, they are favourite of collectors because of their beautiful colours. The mantle is usually ornamented with papillae that provide camouflage and assist in respiration. The colour of the mantle some times matched the spongs it feed upon. (Harasewych, 1991). The Fascinating colour observed in cowries shell can be attributed to various chemical and structural features. Different colours of cowries are dependent on the presence or absence of aluminum compounds and the acidity of the soil (Helman, 2002). The abnormalities usually observed in cowries shell are overproduction of shell in the mantle.

Man has long been inspired by the graceful symmetry and colour of shells. Architecture use shell as design. Greeks and Romans used the shell as part of their building design and decoration (Hayward *et al*., 1996; Hawkins, 2006). The usage of cowries as a type of currency was so strong that the first metal coin in the current Greek colony of Lydia, was introduced around 670BC. (Fish and Fish, 1996; Helman, 2002). Many pharmaceutical product have their origin in seashell. These include Paolin (a drug made from abalone juice) for effective inhibitor of penicillin resis substance. The extraction of bioactive agent of cowries shell is one of the most intensive area of natural product research today yet the field is far from exhaustive.

Cowries shells were used in many area of medicine, examples include deadly venoms of some cowries shells used to help victims of strokes and heart diseases and to produce a revolutionary new drug for chronic pain control (Helman, 2002). The cement of the carrier shell is used as a possible cement for bone fractures. Powdered Pearl's from shell are used as a topical eye medicine and it has been scientifically proved to have some anti-inflammatory effect in painful condition called conjunctivitis and is also used as calcium supplement both for human and animal and is an inhibitor of cancer in mice (Helman, 2002). Report shows that 10% of all cowries had been investigated in detail for bioactive agent (Hayward *et al*., 1996; Fish and Fish, 1996). The present study was undertaken to determine the bioactive compound that contribute to its therapeutic effect.

Materials and Methods

Perforated cowrie shells were purchased locally from the market at Agege, Lagos, Nigeria. The shells were properly washed with sterile water to remove foreign bodies like dust, dirt and insect larva which might have entered during the harvesting from the sea. These samples were broken into small pieces and sundried. Dried samples were grinded with an electric blender into powdery form. The powdered material was stored in a clean dry screw capped bottle at room temperature (37°C) Proximate analysis, mineral composition and phytochemical analysis were carried out on dried samples.

Proximate analysis were carried out according to the procedure of Association of official analytical chemistry (A.O.A.C., 1990)

Mineral composition: The samples were dry ashed at 550°C. The ash was boiled with 10 mL of 20% hydrochloric acid in a beaker and then filtered into a 100 mL standard flask. It was made up to the mark with deionized water. The minerals were determined from the resulting solution using Atomic Absorption spectroscopy (Pye unican Sp9 cambridge, UK).

Phytochemical analysis: Phytochemical screening procedures carried out were adapted from the previous work on plant analysis (Sofowora, 1993).
Determination of alkaloids, 0.5g of the sample was weighed accurately and defatted with 5% ethyl ether for 15 mins. The defatted sample was extracted for 20 mins with 5.0 mL of aqueous HCL in a water bath. The resulting mixture was centrifuged at 3000rpm for 10min to remove filterate (supernatant) 1.0 mL of the filtrate was treated with a few drops of Mayer's reagent and a second 1.0 mL portion was treated similarly with Dragendorff' reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids (Harbone, 1973; Trease and Evans, 1996).

Test for Saponin: Ability of Saponins to produce frothing in aqueous solution was used as screening test for the sample 0.5g of dried extract was shaken with water in a test tube, frothing which persist on warming was taken as evidence for the presence of Saponins.

Test for tannins: 5.0g of dried extract was stirred with 10.0 mL of distilled water. This was filtered and ferric chloride reagent was added to the filtrate. A blue black precipitate was taken as evidence for the presence of tannins (Trease and Evans, 1996).

Test for anthraquinones: 5.0g of dried extract was shaken with 10.0 mL of benzene, this was filtered and 5.0 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet colour in the ammonia cal (lower) phase indicated the presence of free hydroxy anthraquinones (Trease and Evans, 1996).

Test for Cardiac glycosides: 0.5g of dried extract was dissolved in 2.0 mL of glacial acetic acid containing one drop of ferric chloride solution. This was then underlaid with 1.0 mL of concentrated H_2SO_4. A brown ring obtained at the interface indicated the presence of cardenolides.

Results and Discussion

Table 1 shows the proximate composition of perforated cowrie shells (*Cyparica samplomonita*). Moisture and Nitrogen content were found to be very low. However, result also revealed low fat, protein and fibre contents 0.42%±0.03; 5.10%±0.01 and 7.27%±0.01 respectively. This confirms that cowrie is not a good source of fat and protein which are nutritionally important. Carbohydrate is present in considerable amount (14.73%±0.08). The Ash content was found to be high (76.30±0.10). It is a reflection of the total inorganic matter present in a food sample and also indicates that perforated cowrie possess some minerals which are essential for good health.

Table 1: Proximate Composition of perforated cowrie (*Cyparica samplomeneta*) (% dry weight)

Moisture content	0.22±0.11
Ash content	76.30±0.10
Crude fibre	7.27±0.01
Crude protein	5.10±0.01
Nitrogen content	0.82±0.02
Carbohydrate	14.93±0.08
Crude fat	0.42±0.03

Result represent mean±SD of two determinations

Table 2: Mineral Composition of Perforated cowries (mg/100g)

Element	Values
Calcium	91.35±0.45
Magnesium	1.22±0.02
Potassium	0.24±0.01
Aluminium	30.82±0.68
Sodium	29.71±0.03
Manganese	5.40±0.10
Iron	47.52±0.02
Zinc	0.61±0.02

Results are mean ± SD of two determinations

Table 3: Phytochemical analysis data of unripe pulp of *Carica papaya* (dry weight)

Test	Observation
Alkaloid	+ve
Glycosides/cardenolides	+ve
Tannins	+ve
Quinines	+ve

Calcium is the most abundant mineral present in the perforated cowrie shells. The high content of calcium confirms its medicinal role in bone formation. It was reported that the cement of the cowry shell can be used as a possible cement for bone formation (Fish and Fish, 1996) and are used as calcium supplement.
Iron, Aluminum and Sodium are found in reasonable amount (Table 2). Sodium is an extracellular cation involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction. High dietary sodium has been associated with hypertension. Iron is an important trace element in the human body. It plays crucial roles in haemopoiesis, control of infection and cell mediated immunity. The presence of these minerals contributes to its medicinal value.
The presence of phytochemicals like Alkaloids, Glycosides, Tannins, Saponins, Quinones in perforated cowrie shells, (Table 3) which are biologically important contributes to its value in many areas of medicine e.g in physiotherapy and pharmacy.
Cardenolides/Cardiac glycosides are known to be used in the treatment of congestive heart failure (Schneider and Wolfling, 2004).
Saponin inhibits Na^+efflux and activate Na^+-Ca^{2+} antiporter in Cardiac muscle. The increased influx of Ca^{2+} through this antiporter produces elevated cytosolic Ca^{2+} which strengthens the contractions of heart muscle and thereby reducing congestive heart failure. The

presence of cardenolides contributes to the role of deadly venoms of some cowrie shells which are used today to help victims of strokes, heart diseases and produce revolutionary new drug for chronic pain control. Also powdered Pearls from shells are used as topical eye medicine. It has been scientifically proved to have some anti-inflammatory effect on conjunctivitis where the surface of the eye become red and sore. (Fish and Fish, 1996). This report confirms the presence of Alkaloids and Anthraquinones in cowry shells as shown in Table 3.

Conclusion: The present study has shown that cowry shells contain some minerals and secondary plant products which are of biologic importance. The reasonable level of sodium in cowry shell is desirable against the backdrop of reported health effects of high sodium intake. The presence of cardenolides or cardiac glycosides and saponin confirms its therapeutic effects on heart related diseases.

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Effect of Zinc Deficiency on Haematological Parameters and Mineral Contents of Selected Tissues in Albino Rats

O.B. Ajayi
Department of Biochemistry, University of Ado-Ekiti, P.M.B. 5363, Ado-Ekiti, Ekiti State, Nigeria

Abstract: The effect of zinc deficiency on tissue level of some minerals (Na, K, Ca, P, Cu, Fe, Mg, Zn, Mn) in growing rats was investigated. Two groups of rats were fed either a zinc adequate diet or zinc deficient diet (6 ppm) for six weeks. At the end of the feeding period, zinc deficiency decreased the final body weight of the rats by 70% compared to the control. There was no significant difference ($P < 0.05$) in the packed cell volume (PCV) and the white blood cell count (WBC) of the zinc deficient rats was significantly increased ($P < 0.05$) compared to the control. Tissue content of minerals measured in the liver and kidney of both group of rats showed that zinc deficiency significantly increased ($P < 0.05$) tissue concentration of some elements (Fe, Cu, Ma, Ca, K and Na) while Mg and phosphorus levels were lowered compared to the control. This result suggest that zinc deficiency in rats could have adverse effect on intermediary metabolism as a result of interplay in the interactions between these minerals.

Key words: Zinc deficiency, minerals, rat, metabolic function

Introduction

Minerals are inorganic nutrients that are required in small amounts for proper metabolic function in plant and animals. They are obtained through the diet or in combination with organic molecule (Seeley *et al*., 1996). Zinc is an essential element in the nutrition of man and animals and has been identified as an integral of numerous enzyme systems. Zinc status influence several aspects of cellular metabolism. Zinc deficiency has been associated with anaemia, skeletal defects demyelination and lesion in the cardiovascular system (Guthrie, 1989) especially during postnatal development Several studies have highlighted the importance of zinc in nutrition (Prasad, 1991; Adisa and Odutuga, 1998). In general zinc functions to regulate activity of many metalloenzymes (Ajayi and Odutuga, 2004), since it serves as a prosthetic group. The present study is aimed at acquisition of additional information about the effects of zinc deficiency on other minerals in selected tissues of albino rats.

Materials and Methods

Twenty male white albino rats *(Rattus Norvegicus*) were divided into two groups, each containing ten animals and housed in plastic cages of stainless steel wire top and bottom. The animals were acclimatized for 24hours before introducing the experimental diets.

The two groups were maintained on (a) Control diet containing adequate zinc and (b) Zinc deficient diet. The composition of the diet was as described by Odutuga and Ajayi (1998). All reagents were of analytical grade and products of BDH Limited and Sigma chemical Company, England. The feeding trial lasted six weeks.

Tissue preparation: The rats were anaesthetized and sacrificed by cervical dislocation. Blood was drawn from the heart by cardiac puncture using a sterile syringe and needle. Blood was drawn into heparinized capillary tube for PCV analysis. Blood serum was obtained and stored for haematological parameters. The liver and kidneys (decapsulated) were removed, drained of blood and weighed .

Each tissue was ashed and dissolved in 10% HCl and made up to 100ml standard flask with distilled water. The mineral content of each tissue were analyzed using Atomic Absorption Spectrophotometer (AOAC, 1990). Phosphorus was determined calorimetrically using vanadonomolybdate method.

Results and Discussion

Table 1 shows the mean initial and final body weight of rats in the control and zinc deficient rats. In the present study the average weight gained by the zinc deficient group of rats was 70% less than that of the control respectively. The overall growth pattern showed that zinc deficient rats grew less than the control. It may be due to the fact that zinc plays an essential role in the formation of DNA and RNA and hence synthesis of protein. (Prasad, 1991). Hence a low- zinc status may affect protein metabolism which can result in reduced growth rats. This observation agrees with previous reports (Odutuga and Ajayi, 1998). Iron and copper deficiencies had also been shown to affect growth of rats (Oloyede and Folayan, 1995: Ajayi, 2005) Also zinc deficiency had been associated with reduced blood growth hormone levels in animals and humans (King, 1990)

Table 2 shows the PCV and WBC count of the zinc deficient and control rats.

There was no significant difference between the packed

Table 1: Mean Initial and final body weight of animals

	A	B
Final Body weight (g)	88.84±2.32	63.10±1.20
Initial Body weight (g)	40.73±4.31	35.1±2.10

A: Control rats, B: Zinc deficient rats

Table 2: Haematological Parameters

	Packed cell volume (PCV) %	White blood cell WBC (count).
A	44.50 ± 5.6	2950 ± 70.71
B	46.50 ± 2.12	4150 ± 21.23

Table 3: Mineral contents of the Kidney

Minerals	Control (ppm)	Zinc deficient (ppm)
Iron	40.13±18.73	82.59±21.61
Copper	24.55±5.87	49.73±5.07
Zinc	229.42±1.39	225.93±1.89
Manganese	26.12±8.02	115.93±4.23
Magnesium	53.27±13.24	3.73±1.36
Calcium	41.47±13.58	80.80±28.82
Potassium	411.43±74.96	530.778±21.77
Sodium	497.76±76.85	862.10±148.85
Phosphorus	165.40±11.50	88.76±46.94.

Table 4: Mineral Contents of the Liver

Minerals	Control (ppm)	Zinc- deficient (ppm)
Iron	16.34±3.24	22-24±3.68
Copper	11.90±2.08	13.50±6.85
Zinc	90.07±21.32	90.96±33.12
Manganese	7.43±3.08	5.37±3.10
Magnesium	41.96±7.10	30.33±10.65
Calcium	6.03±3.98	19.13±7.66
Potassium	161.19±26.30	222.12±8.76
Sodium	183.93±27.32	246.22±17.77
Phosphorus	105.72±1.16	88.84±2.94

cell volume (PCV) of zinc deficient rats and the control. This may be due to interference of zinc with copper bioavailability. Relatively low levels of dictary zinc may interfere with copper absorption thereby increasing it and copper helps in the formation of red blood cells (Maurice *et al.,* 1997). The white blood cell count (WBC) for the zinc deficiency rats is significantly higher ($P<0.05$) than the control. This may probably be due to infection since considerable amount of copper facilitates Iron absorption, which favours the multiplicity of invading bacteria (Vander *et al.*, 1998). Also zinc deficiency is characterized by small thymus and spleen with resultant reduction in its capacity for T and β-lymphocyte production (Maurice *et al.*, 1997). Zinc deficiency resulted in accumulation of copper in the liver and kidney of rats. The copper level in these organs were significantly ($P<0.05$) higher than the control. This may be attributed to interference of zinc with copper absorption. Both zinc and copper compete for the same sites on the protein carrier therefore the zinc deficiency is accompanied with excess copper, this is similar to the observation made by (Pfeiffer and Lamola, 1983). The accumulation of copper also suggest the potential for a zinc-copper antagonism affecting metabolic phenomena. Its been reported that the intestinal mucosa is the site of competition between these closely related transition metals. The magnesium content of the zinc deficiency rats was significantly lower than the control. The reduction poses a threat to life because magnesium serves as a co- factor of many enzyme especially those that are energy yielding (ATPases) (Nelson and Cox, 2002). The low magnesium content herein observed in these organs may have adverse effect on intermediary metabolism.

The reason for the significant reduction ($P<0.05$) in manganese content of these organs cant be understood, because zinc deficiency had no known effect on the intestinal absorption of manganese in the rat. Nevertheless manganese serves as a cofactor to many enzymes (Wall Work *et al.*, 1982.). Magnesium is also needed for the O- methylation of catecholamines norepinephrine, epinephrine and dopamine to methylated derivatives.

The iron content of the liver and kidney were significantly increased ($P<0.05$) compared to the control. This may be due to the considerable amount of copper in the zinc deficient rats, since copper is needed to facilitate the absorption of iron (Maurice *et al.*, 1997).

The calcium content in these organs is significantly higher ($P<0.05$) than the control. This may likely affect the synthesis of parathyroid hormone which promotes intestinal absorption of calcium and demineralization of bone leading to increase in extracellular calcium. This may in part be responsible for the neuromuscular changes observed in zinc deficient rats (Prasad, 1991).

The sodium and potassium content of the zinc deficient group is significantly higher than that of the control. Sodium is found in soft tissues and extracellular fluids while potassium is the major constituent of intracellular fluids required for the maintenance of cell integrity. Both are concerned with maintenance of acid- base balance and osmotic regulation of body fluids (Willard *et al.,* 1989). The significant increase in the concentration of these two may probably affect the activity of enzymes involved in active transport in the kidney especially the sodium pump may be adversely affected.

The phosphorus content is zinc deficiently rats is significantly lower ($P<0.05$) in these organs. Phosphorus status may affect the level of the cellular currency (ATP) of the body. The finding in this study corroborates earlier reported that the less phosphate groups would be available for the phosphor of ethanolamine and choline needed for the synthesis of phosphatidyl ethanolamine and phosphatidyl choline due to reduced activity of Alkaline phosphatase in zinc deficiency (Odutuga and Ajayi, 1998).These two major phospholipids play vital roles in the maintenance of membrane fluidity. A change in membrane fluidity is an indication of membrane damage (Odutuga, 1977; Cunnane, 1988). The zinc content in the liver is not

significantly different from the control while a significant difference ($P<0.05$) was observed for the kidney this could be due to the fact that liver acts as zinc pool, (or as zinc stores), while the kidney turns over zinc rapidly during zinc deficiency. Its been reported that some organs bind zinc more strongly than others. (Kings, 1990). From the foregoing, zinc deficiency in rats could grossly affect intermediary metabolism since most of these minerals serve as activators to various enzymes involved in metabolic pathways.

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Effects of Conjugated Linoleic Acid on Body Composition and Selected Biochemical Parameters in Obese Women

Habibe Sahin[1], Fatma Uyanik[2] and Neriman Inanc[1]
[1]Department of Nutrition and Dietetics, Ataturk Health School, University of Erciyes, 38039, Kayseri, Turkey
[2]Department of Biochemistry, Faculty of Veterinary, University of Erciyes, 38090, Kayseri, Turkey

Abstract: This study was performed to investigate the effects of conjugated linoleic acid (CLA) on body composition and selected biochemical parameters in obese women. Twenty women, aged between 22 and 48 years, with body mass index (BMI, kg/m^2) over 25 received 1.8 g CLA/day for 8 weeks. Basal metabolic rate (BMR), anthropometric and selected biochemical parameters as well as serum insulin, leptin and ghrelin were measured at baseline and 8 weeks. Significant decreases were found in body weight, body mass index (BMI), waist and hip circumferences ($p<0.05$) while no effect of CLA was determined on BMR, waist/hip ratio, fat content and lean body mass (LBM). Significant decreases were found in serum triglycerides (TG), total cholesterol (TC) ($p<0.05$), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and plasma leptin ($p<0.01$) levels. Serum glucose level decreased but remained within normal range while insulin levels of subjects increased ($p<0.01$). Slight but not significant increases were found in plasma ghrelin and serum high density lipoprotein (HDL) levels. The results of this study have shown that supplementation of 1.8 g CLA/day for 8 weeks affect lipid and carbohydrate metabolisms and reduce body weight, waist and hip circumferences which are the indicators of abdominal obesity that is a risk factor for coronary heart disease.

Key words: Anthropometric measurements, conjugated linoleic acid, obesity

Introduction

Obesity, a chronic condition will most likely be needed a long term treatment (Udall *et al*., 2005). Dietary supplements to treat obesity appeal to many patients who desire a 'magic bullet' for weight loss (Saper *et al*., 2004). Approximately 50 individual dietary supplements and more than 125 commercial combination products are available for weight loss (Lenz and Hamilton, 2004). Conjugated linoleic acid (CLA) compounds are considered as such products (Joyal, 2004). Conjugated linoleic acid is a group of isomers conjugated octadecadienoic acid that occur naturally in foods, mostly in dairy products (Terpstra, 2004). Ritzenhaler *et al*. (2001) reported that the intake of total CLA as measured with the food duplicate method was 212 mg/day for men and 151 mg/day for women and 60% of the CLA intake was derived from dairy products and 37% from meat products. The cis-9, trans-11 CLA isomer, also called rumeric acid, accounted for >90% of the total CLA intake. Commercial CLA preparations are produced by isomerization of linoleic acid and contain predominantly cis-9, trans-11 and trans-10, cis-12 octadecaenoic acid in a 1:1 ratio (Terpstra, 2004).

The body fat-lowering effect of CLA in experimental animals has led to the idea that CLA could be used as a tool in body weight management in humans (Park *et al*., 1997, Yamasaki *et al*., 2003, Park and Pariza, 2001). However, consistent and conflicting effects of CLA on body composition have been documented in human (Blankson *et al*., 2000; Zambell *et al*., 2000; Riserus *et al*., 2002).

In some previous studies (Blankson *et al*., 2000; Smedman and Vessby, 2001), CLA has been shown to reduce body fat in human in contrast, no effects of CLA on body composition were reported in some other studies (Zambell *et al*., 2000; Riserus *et al*., 2002). Similarly, inconsistent results were reported concerning the effects of CLA on biochemical parameters related to lipid and carbohydrate metabolisms (Riserus *et al*., 2002; Smedman and Vessby, 2001). Furthermore, to the author's knowledge, there is no study investigating the effects of CLA on obesity related hormones such as ghrelin and limited studies on leptin (Medina *et al*., 2000; Nagao *et al*., 2003). Thus, the metabolic effects of CLA are not clear yet. The effects of CLA in human have been also investigated mostly in the people who were on a controlled diet or exercise programmes (Zambell *et al*., 2000). Therefore, this study was performed to investigate the effects of CLA on body composition and on recently discovered hormones related to obesity as well as other biochemical parameters related to carbohydrate and lipid metabolisms in obese women who were neither on a diet nor on an exercise programme.

Materials and Methods

Subjects: This study was conducted at Erciyes University, Kayseri, Turkey between June and August

Table 1: Effects of conjugated linoleic acid supplementation on basal metabolic rate and anthropometric measurements of obese women

	Baseline	After CLA supplementation	P
BMR (kcal)	1533±37.22	1540±35.36	-
Weight (kg)	78.54±3.22	75.38±3.74	*
Height (cm)	161.08±1.13	161.08±1.13	
BMI (kg/m^2)	30.41±1.50	29.05±1.75	*
Waist circumference (cm)	94.58±2.91	90.58±4.01	*
Hip circumference (cm)	114.50±3.23	111.58±3.63	*
Waist/hip ratio	0.81±0.01	0.80±0.02	-
Body fat (%)	38.16±2.03	36.13±3.17	-
LBM (%)	62.06±2.11	63.71±3.14	-

*p<0.05

2005. Twenty female subjects with BMI (kg/m^2) over 25 were included in the study. Subjects were between 22 to 48 years of age, pre-menopausal, not taking any dietary, vitamin and mineral supplements, not on weight loss and/or exercise programmes and with no history of chronic or acute diseases. All subjects gave written and verbal informed consent. Subjects were asked not to alter their own dietary habits and life style during the course of the study except taking the CLA supplement. This study was approved by The Ethics Committee of Faculty of Medicine, University of Erciyes (Approval date and no. 06.07.2004, 04/240).

Experimental design: The subjects were asked to take 3 capsules/day during the study. All measurements were conducted baseline and after CLA supplementation. The subjects received gelatine capsules containing 600 mg CLA per capsule (Fatty acid specification C18:2, conjugated (CLA) relative area 80-84 %, C18:2 conjugated c9, t11 (CLA isomer) relative area 37-42%, C18:2 conjugated t10, c12 (CLA isomer) relative area 37-42 %, Skip, Sweden). The baseline values and changes after suplementation were compared.

Anthropometric measurements: Body weight and height were measured and BMI of subjects were calculated. Waist and hip circumferences of subjects were measured three times and waist/hip ratio was calculated with the mean value of three measurements. Body fat content (%), LBM (%) and BMR (kcal) were measured by Bioelectrical Impedance (Bodystat 1500 Tanita). Subjects avoided coffee and alcohol consumption as well as excess physical activity before the blood sampling and body composition measurements.

Sample collection: Venous blood samples were collected after overnight fasting. Blood samples were incubated 1 hour at room temperature and sera were separated then stored at -20°C until biochemical analysis. Blood samples with EDTA Na_2 were immediately centrifuged and plasmas were separated, stored at -70°C until leptin and insulin analysis. For ghrelin analysis, whole blood was directly drawn into centrifuge tubes that contain 500 IU of aprotinin and 1.25 mg of EDTA Na_2 per 1 ml of blood and samples were kept on ice to prevent/minimize the breakdown of ghrelin and tubes were immediately centrifuged at 1500 g for 15 minutes at 4°C for separating plasma. Then 10 µL of 1 mol HCl was added to per ml of plasma and plasma samples were stored at -70°C until ghrelin analysis.

Biochemical analysis: Serum glucose, (Chema Diagnostica, Italy), TG, TC, HDL (Valtek, Chile) concentrations were determined with commercial kits by a Shimadzu 1208 UV/VIS spectrophotometer. The HOMA index which, is an indicator of insulin resistance, was calculated by the following formula described elsewhere. HOMA = Fasting insulin concentration (µU/mL) X Fasting glucose concentration (mmol/L)/22.5 (Matthevs *et al*., 1985).

Low density lipoprotein concentration was calculated using the Friedewald's formula (LDL = TC-(HDL+TG/5)) and VLDL concentration was calculated using the formula TG/5 (Mahley, 1993). Plasma insulin (Roche Elecsyma E-170, Roche Diagnostics D 68298 Mannheim, U.S.A.), leptin (Mouse Leptin ELISA kit, Linco Research Inc., Missouri, U.S.A.) and ghrelin (Active Ghrelin ELISA kit, Linco Research Inc., Missouri, U.S.A.) concentrations were determined with commercial ELISA kits by Modular Analytics E 170 Module, Roche Diagnostics (Indianapolis, U.S.A.).

Statistical analysis: Data were analyzed by SPSS 10.0 version for Windows. Paired sample t test was used for determination of the difference between the baseline values and values determined after CLA supplementation. The level of significance was set at 0.05 for overall statistical analyses. Data were expressed as means ± standard error of means (SEM).

Results

Significant decreases were found in body weight, BMI, waist and hip circumferences ($p<0.05$) after CLA supplementation. Conjugated linoleic acid had no effect on BMR, waist/hip ratio, fat content (%), LBM (%) (Table 1). Conjugated linoleic acid reduced TG, TC, LDL, VLDL ($p<0.05$) and leptin levels ($p<0.01$). Serum glucose level decreased but remained within normal range while insulin levels of subjects increased ($p<0.01$). Slight but not significant increases were found in plasma ghrelin and serum HDL levels (Table 2).

Discussion

Many studies have been performed to demonstrate the effect of CLA on body composition but inconsistent results were obtained (Blankson *et al*., 2000; Zambell *et al*., 2000; Smedman and Vessby, 2001; Matthevs *et al*., 1985). In this study, 1.8 g CLA/d reduced body weight and BMI significantly but had no effect on BMR as in the

Table 2: Effects of conjugated linoleic acid supplementation on leptin, ghrelin and selected biochemical parameters of obese women

Parameters	Baseline	After CLA supplementation	P
Triglycerides (mg/dl)	116.13±12.62	97.72±12.62	*
Total cholesterol (mg/dl)	180.06±9.54	158.97±8.60	*
HDL (mg/dl)	61.60±6.94	63.95±5.55	-
LDL (mg/dl)	95.23±10.62	75.47±12.27	†
VLDL (mg/dl)	23.22±2.52	19.54±1.61	*
Glucose (mg/dl)	82.98±3.37	73.99±4.22	*
Leptin (ng/ml)	36.82±3.24	29.80±3.51	†
Ghrelin (fmol)	15.74±1.86	17.45±3.92	-
Insulin (mg/dl)	8.24±1.59	14.34±2.89	†
HOMA	1.71±0.33	2.52±0.46	†

* p<0.05. † p<0.01

study of Zambell *et al.* (2000) who suggested no effect of 3 g CLA/d on body composition and energy expenditure in adult women.

Smedman and Vessby (2001) found a 3.8 % of reduction in body fat and no changing body weight, BMI and abdominal diameter after 12 week of treatment with 4.2 g CLA/day and Blankson *et al.* (2000) also determined reductions in body fat with 3.4 g and 6.8 g CLA/day in obese subjects. However, in the present study, although statistically not significant, a slight reduction occurred in body fat with 1.8 g CLA/day.

Lack of the effect of 1.8 g CLA consumption on LBM in the present study confirms the results of Blankson *et al.* (2000) who observed no significant difference in LBM with the consumption of CLA between 1.7 g and 6.8 g/day in obese subjects.

Statistically significant decreases were observed in waist and hip circumferences, which are the indicators of abdominal obesity (Misra *et al.*, 2005). Regional body fat distribution has an important influence on metabolic and cardiovascular risk factors (Carr and Brunzell, 2004). Increased abdominal fat accumulation is strongly related to clinical as well as subclinical coronary heart diseases (Nasir *et al.*, 2005). Waist circumference can be used as a complementary measurement to identify health risks (Wannamethee *et al.*, 2005). In the present study, none of the subjects had clinic or subclinic history of coronary artery diseases, but they were still under risk of such diseases because they were obese (Joyal, 2004; Bhattacharya *et al.*, 2005). The reduction in waist and hip circumferences thus the reduction in abdominal obesity and TG, TC, LDL, VLDL and despite being not statistically significant, a slight increase in HDL may show that CLA reduces risk of coronary heart disease.

In a study of Riserus *et al.* (2002), 3.4 g of CLA supplementation tended to increase insulin concentration as in the present study, in contrast to the finding of these authors, serum glucose concentration decreased but it was still within the normal range. The high HOMA index determined in this study was paralleled with \the increased insulin level. The HOMA index is accepted an indicator of insulin resistance with the cut off point of 2.5 for adults (Matthevs *et al.*, 1985). It may be speculated from these findings that CLA may have some undesirable effects such as insulin resistance and increased plasma insulin (Terpstra, 2004).

Ghrelin is a recently identified, a 28 amino acid peptide, which is produced mainly in the stomach and circulates in blood (Akamizu *et al.*, 2004). Ghrelin an orexigenic hormone (Muccioli *et al.*, 2002) that may be involved in body weight regulation (Cummings *et al.*, 2002), is reduced in obesity (Morpurgo *et al.*, 2003, Soriano-Gullen *et al.*, 2004; Tschop *et al.*, 2001). It has been suggested that circulated ghrelin concentration is influenced by body fat distribution (Malik *et al.*, 2004) and negatively correlates with body fat (Fagerberg *et al.*, 2003). In this study, because CLA caused no effects on body fat content, the lack of effect of CLA on ghrelin concentration is not surprising.

Leptin is secreted by adipocytes in proportion to the amount of lipid stored and may act as a signal of body energy stores to the brain (Terpstra, 2004). Leptin concentrations in humans are increased with obesity (Bennett *et al.*, 1997). Fat tissue proportion can be measured accurately by determining the blood leptin concentration (Bates *et al.*, 2004). Medina *et al.* (2000) and Riserus *et al.* (2002) found no effects of the consumption of CLA for 9 and 12 weeks on leptin concentration in human. However, in this study, reductions in blood lipid and leptin levels, body weight and although not significant reduction in body fat confirm the existence of a relationship between blood leptin concentrations and body fat proportion (Shibata *et al.*, 2003; Bates and Myers, 2004; Maffei *et al.*, 1995).

In summary decreases in body weight, waist and hip circumferences, blood lipid and plasma leptin levels and slight decreases in body fat may show that supplementation of CLA for 8 weeks improves body composition and reduce abdominal obesity in obese women.

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Effect of Zinc Sulfate Supplementation on Lipid and Glucose in Type 2 Diabetic Patients

Mohammad Afkhami - Ardekani, Mahdi Karimi, Seid Mohammad Mohammadi and Forough Nourani
Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Abstract: Type 2 diabetes mellitus is a chronic, progressive illness that causes considerable morbidity and premature mortality. More people are still having tendency to use herbal or alternative remedies. Zinc is a trace mineral which may be effective in diabetic patients. We evaluated the effect of zinc sulfate on biochemical markers of type 2 diabetic patients. In a randomized, controlled trial on diabetic subjects, forty patients received randomly either 660mg zinc sulfate or placebo for six weeks. Body Mass Index, Blood Pressure, Fasting Blood Sugar, 2-h postprandial glucose, Glycated hemoglobin, Triglyceride, cholesterol, low - density lipoproteins, high - density lipoproteins were checked before and six weeks after beginning of the study. HbA1C, BMI and Blood Pressure were measured after 12 weeks to evaluate the long term effect of drugs. The mean age of patients was 52.67±8.60. level of FBS, 2HPP, HbA1C decreased after six week treatment with zinc sulfate but it was not statistically significant. Due to zinc sulfate administration, significant decrease occurred in TG (P=0.005), chol (p=0.02), LDL (0.01) and systolic blood pressure (p=0.02). HDL was increased but it was not significant. No statistically significant differences were found prior to and after zinc treatment in BMI and diastolic blood pressure. After 12 weeks, there was a significant decrease in HbA1C (P=0.04) with zinc sulfate consumption. Zinc sulfate consumption in addition to other nutritional and pharmacological treatments in type 2 diabetic patients could be effective in lipid profile.

Key words: Zinc sulfate, Type 2 diabetes mellitus, lipid profile

Introduction: Type 2 diabetes mellitus is a polygenetic disorder resulting from interaction of both hereditary and environmental factors (Jinlin *et al*., 2007). It is a chronic, progressive illness that causes considerable morbidity and premature mortality (Kleefstra *et al*., 2007. Amos *et al*., 1997). The worldwide prevalence of type 2 diabetes is high and is increasing steadily (King *et al*., 1998). Approximately, 150 million people worldwide are affected by type 2 diabetes mellitus and this figure is expected to double in the next 20 years (Freeman and Cox, 2006). Prevalence of type 2 diabetes is 13.8% in Yazd province, Iran (Afkhami-Ardekani *et al*., 2001). Type 2 diabetes is associated with the increased risk of microvascular and macro vascular complications (Nazimek-Siewniak *et al*., 2002). Zinc is known to be an essential trace mineral which is necessary for health and growth and is also essential for the function and activity of over 200 metalloenzymes (Chen *et al*., 1991). The ability of zinc to retard oxidative processes has been recognized for many years (Powell, 2000). Zinc is an essential mineral that is required for various cellular functions. Its abnormal metabolism is related to certain disorders such as diabetic complications (Song *et al*., 2005).

Abnormal zinc and lipid plasma levels occur more frequently in metabolically uncontrolled diabetic patients. Yet, zinc sulfate supplementation may be a therapeutical resource to recover some functioning and improve life span (Partida-Hernandez *et al*., 2006).

This article reports the effect of zinc sulfate on glucose and lipid profile of type 2 diabetes mellitus patients.

Materials and Methods

The study design was randomized, controlled trial. Subjects enrolled from Yazd Diabetes Research Center, Yazd, Iran. Inclusion criteria included type 2 diabetic patients with fixed drug dosage in past 6 months, fixed weight in past 3 months, without taking vitamins or mineral supplements in the previous 2 months and without clinical involvement of kidney, heart and lung. The subjects were fully informed of the purpose, procedures and hazards of trial and were free to leave the trial at any time. Written informed consent was obtained from all participants. The research protocol was approved by the ethics committee on human experimentation of Yazd University of Medical Sciences. Forty subjects divided randomly into two groups and supplemented daily with 660 mg zinc sulfate or placebo for six weeks. Zinc sulfate was manufactured by ALHAVI Company.

Subjects were instructed not to modify diet or activity level; each individual maintained dietary records at intervals throughout the experiment. Body Mass Index (BMI), Blood Pressure and biochemical markers included Fasting Blood Sugar (FBS), 2-h postprandial glucose (2hpp), Glycated hemoglobin (HbA1C), Triglyceride (TG), cholesterol (chol), low -density lipoproteins (LDL), high - density lipoproteins (HDL), Blood Urea Nitrogen (BUN), Creatinin (Cr), Alanine aminotransferases (ALT),Aspartate aminotransferases, (AST) were checked before the beginning of the study. BMI was calculated as the weight in kilograms per the

square of height in meters and blood pressure measured with the person in the sitting position after a 5-min rest. All blood specimens were drawn at 8 a.m. after a period of 8 hours fasting. All patients were examined carefully and depending upon the treatment groups, each subject received drugs for a period of six weeks. Subjects eat zinc sulfate every eight hours by meal with a large glass of water (220 mg TDS).

BMI, blood pressure, AST, ALT and drug complications such as nausea, vomiting, abdominal pain, diarrhea, constipation, reduction of appetite were checked after 3 weeks.

At the end of 6 weeks all the indices checked as before the beginning and the drug complications were asked as well. HbA1C, BMI, blood pressure and complications were analyzed after 12 weeks to evaluate the long term effect of drugs.

Statistical analysis: All statistical analyses were performed by using SPSS, version 11.50. Data of continuous variables are expressed as means ±standard deviation. Differences between groups were assessed by the paired t test.

Results

All subjects completed the study. The mean age of patients was 52.67±8.60 with male constituting 40% of patients (16 male and 24 female). Mean duration of diabetes was 7.07±4.94 years. 17.5% and 22.5% of diabetic patients had hypertension and hyperlipidemia respectively. 65% and 62.5% ate metformin and glybenclamide respectively but only 5% had acarbose consumption. The characteristic and baseline biochemical markers of subjects are shown in Table 1.

As it is seen in Table 2, level of FBS, 2HPP, HbA1C decreased after six week treatment with zinc sulfate but it was not statistically significant.

Due to zinc sulfate administration in diabetic patients, a significant decrease occurred in TG (P=0.005), chol (p=0.02), LDL (0.01) and systolic blood pressure (p=0.02).

Table 1: Characteristic and baseline biochemical markers of subjects

Variables	Mean±SD
BMI (kg/m^2)	27.60±5.92
FBS (mg/dl)	156.10±50.30
2hpp (mg/dl)	221.85±81.89
HbA1C (%)	7.83±1.53
TG (mg/dl)	216.92±113.92
Chol (mg/dl)	170.97±44.29
LDL (mg/dl)	92.45±34.63
HDL (mg/dl)	47.92±15.30S
BP (mmHg)	122.92±25.74
DBP (mmHg)	73.25±11.00

Body Mass Index (BMI), Fasting Blood Sugar (FBS), 2-h postprandial glucose (2hpp), Glycated hemoglobin (HbA1C), Triglyceride (TG), cholesterol (chol), low - density lipoproteins (LDL), high-density lipoproteins (HDL), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP).

HDL was increased but it was not significant (p=0.14). No statistically significant differences were found prior to and after zinc treatment in BMI and diastolic blood pressure.

Only two patients had mild abdominal pain. After 12 weeks, there was a significant decrease in HbA1C (P=0.04) in zinc sulfate group but a significant decrease didn't occur in BMI and blood pressure.

As it is seen in Table 2, no statistically significant differences were found prior to and after six weeks placebo treatment in different biochemical variables.

Discussion

Recently, diabetic patients have tendency to use complementary and alternative medicine beside routine therapies. Zinc is one of the minerals used by diabetic patients. Anderson *et al.* (2001) study on 110 type 2 diabetes mellitus with HbA1C > 7.5% revealed that more than 30% of the subjects may have been zinc deficient (Anderson *et al.*, 2001).

In present study, no statistically significant differences were found prior to and after 6 weeks zinc sulfate treatment in FBS, 2hpp, HbA1C, HDL, BMI and diastolic blood pressure.

Table 2: Biochemical parameters before to and after Zinc sulfate versus placebo consumption

	Zinc sulfate			Placebo		
Variables	Pre-trial	Post-trial	P-value	Pre-trial	Post-trial	P-value
BMI(kg/m2)	28.33±6.6	26.75±5.08	0.16	26.86±5.15	26.75±5.08	0.16
FBS (mg/dl)	150.35±64.02	134.25±57.81	0.09	157.60±31.08	159.50±27.35	0.55
2hpp (mg/dl)	186.65±78.57	174.70±74.28	0.06	259.85±68.45	250.55±60.85	0.05
HbA1C (%)	8.13±2.03	7.35±1.62	0.05	7.53±0.71	7.46±0.73	0.62
TG (mg/dl)	227.85±130.61	138.30±75.43	0.005*	206±96.58	197.15±99.41	0.21
Chol (mg/dl)	161.05±47.82	126.40±33.27	0.02*	80.90±39.16	177.85±38.59	0.29
LDL (mg/dl)	85.85±38.39	56.55±21.08	0.01*	99.05±29.95	95.80±28.68	0.10
HDL (mg/dl)	49.50±10.17	56.80±21.85	0.14	46.35±19.28	46.55±19.26	0.51
SBP (mmHg)	134.25±18.48	129.50±18.48	0.02*	112.50±6.38	106.10±23.50	0.26
DBP (mmHg)	76.75±13.79	71.5±9.33	0.1	76.75±13.79	71.50±9.33	0.10

Body Mass Index (BMI), Fasting Blood Sugar (FBS), 2-h postprandial glucose (2hpp), Glycated hemoglobin (HbA1C), Triglyceride (TG), cholesterol (chol), low-density lipoproteins (LDL), high-density lipoproteins (HDL), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP). *Statistical significance when p<0.05.

Due to zinc sulfate administration in diabetic patients, a significant decrease occurred in TG (p=0.005), chol (p=0.02), LDL (p=0.01) and systolic blood pressure (p=0.02). After 12 weeks, there was a significant decrease in HbA1C (p=0.04).

Some investigations indicated that a zinc-enriched diet has beneficial effects on basal and postprandial glycaemia, the content of cholesterol and triglycerides (Ghayour-Mobarhan *et al.*, 2005).

In Roussel *et al.* (2003) study on 56 diabetic patients (divided to zinc gluconate and placebo group) treated with 30 mg zinc gluconate, HbA1c decreased from 8.9±0.4 to 7.7±0.3% following six months of zinc supplementation, but decreases were not significant and no changes was seen in FBS (Roussel *et al.*, 2003).our result about FBS and HBA1C was similar to it.

Cunnane (1988) believed that zinc intimately affects many aspects of lipid metabolism through established enzymes but also has modulator effects whose mechanism is not obvious or established. In individuals with type 1 diabetes mellitus receiving 30 mg of zinc as zinc gluconate for three months, there were decreased lipid peroxidation and improvement in antioxidant status (Faure *et al.*, 1995; Faure *et al.*, 1993).

In Partida-Hernandez *et al.* (2006) survey on type 2 diabetic patients who received 100 mg zinc sulfate, there was no statistically change in FBS and HBA1C after 12 weeks (Partida-Hernandez *et al.*, 2006). In our survey HBA1C was statistically decreased after 12 weeks which may be related to the higher dosage of zinc sulfate in our study.

The diabetic patients had changes in their lipid profile after a 12-week zinc treatment as compared with placebo treatment in Partida-Hernandez *et al.* (2006) survey. The 100 mg zinc sulfate treatment was well tolerated, significantly reduced total cholesterol (p=0.01) and triglyceride concentrations (0.02) and increased those corresponding to zinc as well as HDL cholesterol in the bloodstream (0.002) but decrease in LDL was not significant (0.22) (Partida-Hernandez *et al.*, 2006). Differences with our study is related to drug dosage.

Results of randomized controlled trials of Hughes and Samman (2006) show that LDL, total cholesterol and triglycerides in plasma are unaffected by supplementation with up to 150 mg Zn/d. In contrast, HDL concentrations decline when zinc supplements provide a dose >50 mg/d (Hughes and Samman, 2006). Higher dose of zinc sulfate is needed to decrease the TG, total cholesterol and LDL similar to our study.

Hooper *et al.* (1980) study examined the effect of zinc administration on serum lipoprotein values in man. Twelve healthy adult men ingested 440 mg of zinc sulfate per day for five weeks. High-density lipoprotein-cholesterol concentration decreased 25% below baseline values (40.5 to 30.1 mg/dL). Total cholesterol, triglyceride and low-density lipoprotein-cholesterol levels did not change throughout the study (Hooper *et al.*, 1980). In Roussel *et al.* (2003) study which indicated previously, no changes was seen in lipid profile. (Roussel *et al.*, 2003).

In Freeland-Graves *et al.* (1982) study, four levels of zinc supplements (0, 15, 50, or 100 mg/day) were given to 32 women for 8 weeks. No significant differences were seen in HDL-cholesterol over the 8 week except in the 100 mg group at week 4 (Freeland-Graves *et al.*, 1982).

In conclusion, it seems that zinc is a proper mineral in diabetic patients due to their deficiency and consumption of zinc sulfate in addition to other nutritional and pharmacological treatments in type 2 diabetic patients could be effective in lipid profile.

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Nutritional Evaluation of a Dehydrated Shredded Meat Product, (Danbunama)

O.O. Ogunsola and A.B. Omojola
Department of Animal Science, Meat Science Laboratory, University of Ibadan, Ibadan, Nigeria

Abstract: The qualitative effects of different oil types used in processing of danbunama, a dehydrated shredded meat product in relation to its palatability, physical, chemical and nutritional attributes are the focus of this study. The effect of different oil types on the sensory qualities of the product was carried out using semitendinosus part of beef. The three different oil types used, decolorized palm oleic oil (T_1) local bleached palm oil (T_2) and pure groundnut-oil (T_3) were subjected to lipid oxidation analysis to determine the Thiobarbituric acid value (TBA) peroxide and acid values at 1st, 3rd, 6th and 9th weeks of storage. Sensory evaluation showed that there were no significant differences amongst the oil types used on the parameters tested for. The proximate analysis of danbunama determined showed its moisture range to be 4.22-4.50%, crude protein% range of 38.9-43.5%, T_1 differed significantly from that of T_2 and T_3, the crude fat% differed significantly for all oil types. Lipid oxidation in T_3 (0.70±0.01) was significantly higher at week 6 while T_1 (0.81±0.01) recorded a significantly higher value at week 9. Danbunama can be prepared from any of the oil types and with proper packaging, the nutritional status of the product at week 1 does not significantly differ from that at week 9. The product is a nutritive meal or snack, easy to carry requiring no sophisticated packaging and is quite stable at room temperature. Rancidity will not pose a treat if good quality raw materials are utilized during processing.

Key words: Danbunama, oil - types, storage, nutrition and rancidity

Introduction

The term intermediate moisture meat product is used to describe meat product that have less than 20% of moisture present in it after it has been processed with any of the preservation methods. Danbunama, is an intermediate moisture meat product processed by cooking, pounding and then pan frying with addition of spice. It is peculiar to the Northern part of Nigeria. It is processed principally from semitendinosus or part of cattle (beef). This meat product is commonly consumed by the elites of the Hausa communities found in Nigeria and is usually served at parties. It is a meat product that developed as a means of preserving cooked meat in the absence of facilities for refrigeration storage by the Hausa people. It is a meat product that has good nutritive value and relative shelf stability at room temperature. It can serve as a snack or combined with other food as part of daily diet for the general populace. It also gives convenience to travelers and campers because of its advantages of being light weighted, easy-to-pack and ready-to-eat. (Ockerman and Li, 2000).

Danbunama has its ally in other cultures: The meat floss, made from lean pork meat called Machana in Mexico, a combination of cured meat and floss snacks (called "niu rou kang") is a product of Shanghai China. Travelers to these countries abroad have access to buy these meat products and several other meats snacks in their general markets, super markets and stores. (Chang *et al*., 1996).

Due to the fear consumers have about fat intake and cholesterol, frying as a method of processing meat is not very popular amongst meat processors. The oil types used for frying in this work were subjected to various test of its quality. The locally bleached palm oil, pure groundnut oil and refined vegetable oil were used for the experiment to determine their safety in the production of danbunama.

Materials and Methods

The processing of danbunama: For each preparation of danbunama 2kg semitendinosus muscle part of matured male cattle was used, this was trimmed to remove any surface dirt and visible fat, cut into small chunks of average weight of 140g, rinsed once and boiled with 2 or 3 medium sized onions (equivalent to 180g) and a little quantity of water that would cook the meat leaving very little stock (the meat must not be submerged in water). The cooking was done at 100°C, for $2^1/_2$hours to the point when the meat was thoroughly cooked such that it would break when pressed with the thumb and the forefinger. The cooked meat was transferred into the mortar (a local wooden device for pounding food), thinly sliced onions were added, a level teaspoon of spice mixture (5g) was added, pounding (with the pestle) was intensive and consistent on the meat pieces until the meat strands disengaged and were beaten to shreds. The spice mixture consisted of 4 cubes of maggi, 1 level teaspoon salt (5g), added to dried pepper, dried ginger, black pepper and cloves in the ratio of 5:1:1:1.

Table 1: Sensory analysis of prepared shredded meat, danbunama

Parameters	T_1	T_2	T_3
Aroma*	3.67±0.89	3.83±1.27	4.00±0.95
**	4.50±0.52	4.50±0.67	4.50±0.67
Texture*	3.75±1.22	3.00±1.04	3.67±0.98
**	3.83±1.03	3.92±1.08	3.33±1.07
Taste*	3.83±1.19	3.75±1.36	4.00±1.21
**	4.42+0.51	4.50±0.67	4.42±0.67
Juiciness*	3.17±1.11	3.92±0.90	3.42±0.79
**	4.08±1.00	4.00±0.74	4.17±0.58
Overall acceptability*	3.83±0.83	4.0±1.000	3.83±0.94
**	4.08±0.67	4.58±0.67	4.42±0.51

* Values at week 1. ** Values at week 3. T_1 = refined deodorized palm oleic oil (Turkey brand). T_2 = locally bleached palm oil. T_3 = Pure groundnut oil. Means in the same row for same attributes are not significantly different.

The shredded meat was divided into three equal parts, for each oil type that was used for stir-frying of meat at 70 strokes per minutes for 20 minutes (the frying was done such that the pounded meat formed a paste with the oil at the beginning of frying). The three oil types were: The refined decolorized palm oleic oil (Turkey brand) T_1, the locally bleached palm oil, T_2 and the Purified groundnut oil T_3.

After frying each batch of the shredded meat for 20 minutes with continuous stirring, the oil was drained out by application of pressure on the fried pounded meat that was poured into a colander so that the oil easily drained out, thereby preventing the final product from sticking together, leaving a dry and spongy product. The meat was spread out on a tray, allowed to cool, separated into strands and 1 level teaspoon of spice mixture was again added to taste.

Packaging: Danbunama so obtained from the different oil treatments were packaged in airtight containers (polyvinyl chloride bags and plastic containers).

Chemical composition: Moisture, crude fat and protein were determined by the AOAC (1985) methods. The TBA value was determined by photometry method (1944) peroxide value by iodometry method and acid values (AOAC, 1985).

Sensory evaluation: A group of ten panelists cutting across staff and students (aged between 25 and 45) from the University of Ibadan, Ibadan, Nigeria were used to evaluate the shredded meat floss prepared from the oil types. A five point hedonic scale where 1 = dislike a lot and 5 = like very much was used in scoring (IFT, 1981) Organoleptic properties evaluated included aroma, texture, taste, juiciness and overall acceptability. Sensory evaluation was done on freshly prepared danbunama samples.

Statistical analysis: The sensory analysis and other corresponding data were subjected to analysis of variance (ANOVA), SAS and Duncan rating test.

Table 2: Proximate analysis of danbunama from three oil types at week 0

	Nutrient Compositions		
	Moisture	Crude Fat	Crude Protein
T_1	7.37±0.12^a	37.83±0.02^b	41.21±0.01^a
T_2	7.16±0.01^b	35.57±0.05^c	39.64±0.05^b
T_3	6.50±0.01^c	40.85±0.05^a	38.92±0.02^b

Means with different superscripts in the same column are significantly different. ($P < 0.05$)

Table 3: Thiobarbituric acid, peroxide and acid values at 6 and 9 weeks

	Parameters		
	TBA	Peroxide	Acid value
AT 6 WEEKS			
T_1	0.38±0.001^b	1.99±0.141^b	2.76±0.01^a
T_2	0.39±0.040^b	2.84±0.028^a	2.63±0.21^a
T_3	0.50±0.008^a	1.95±0.071^c	2.75±0.02^b
AT 9 WEEKS			
T_1	0.81±0.006^a	1.00±0.028^b	2.89±0.01^b
T_2	0.47±0.018^c	1.05±0.141^b	3.06±0.04^a
T_3	0.70±0.003^b	1.21±0.071^a	3.00±0.02^a

Means with the same superscript along the column are not significantly different ($P < 0.05$). Unit is in ug/g.

Results and Discussion

Results obtained in the study are summarized in Tables 1-3. Table 1 shows the analysis of sensory evaluation conducted at week zero. Organoleptic traits evaluated for did not show any significant differences with oil types used in processing of Danbunama.

The results of the chemical proximate analysis as presented in Table 2, showed that significant differences were observed in the percentage moisture, crude fat and crude protein content of danbunama prepared from the three oil types.

Table 3 gives the thiobarbituric acid (TBA), peroxide and acid values at 6 and 9 weeks.

The profile of the proximate composition of the danbunama produced from the three oil types proved the product to be a very shelf stable product, its low moisture percentage promotes its ability to stay at room temperature in spite of its high level of protein and fat combined. The sensory evaluation of danbunama for aroma, texture, taste, juiciness and overall acceptability showed no significant difference for the different oil types. The taste especially did not seem to differ for all oil types. The fact that danbunama is a relatively new product might be responsible for the insignificant difference in its overall acceptability. Ockerman and Li (2000) in a similar study of pork meat floss, reported that consumers preferred a modified cooking floss to the traditional preparation but reported no significant preference for meat floss with higher level of lard inclusion.

The three parameters evaluated for lipid oxidation were the TBA, peroxide and acid values. The acid value is a

measure of the amount of free acids present in a given amount of oil. It can be overestimated if other acid components are present in the system (such as amino acids in meat). The peroxide values measures the amount of peroxides, which are primary products, formed in the initial stages of oxidation of lipids therefore giving an indication of the progress of oxidation in the oil. The peroxide value was significantly higher in the locally bleached oil. The Thiobarbituric acid value determines the extent of lipid oxidation, the TBA value of 1.0 is considered as the threshold level for rancidity in pork floss (Ockerman, 1985). Thiobarbituric acid reactive substance (TBARS) amount increases with storage days (Pie *et al*., 1990) and inappropriate storage conditions of meat products, together with the action of light and oxygen accelerate oxidation. The peroxide values also increase with storage. During the course of oxidation peroxide values reach a peak and then decline (Yan and White, 1990), the formation of peroxide is the initiation of lipid oxidation. The acid values involve the formation of acids in lipid oxidation this is an indication of hydrolytic rancidity (Table 3) these values also increased markedly with storage.

In conclusion, danbunama in this study was observed to have exceeded the nine-week period, which was the duration of this study. Further work could be carried out to determine exactly at what point of storage that spoilage would set in and how long it takes the oil used in processing to become rancid thus rendering the product inedible. The product properly bottled and kept in a cool place is quite stable at room temperature.

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Effect of Groundnut Paste on the Quality of Maize Based *Masa*

J.A. Ayo, H. Agu, V.A. Ayo and O.F. Famoriyo
Department of Food Science and Technology, The Federal Polytechnic, Bauchi, Nigeria

Abstract: The maize grains were dehulled, washed, dried and milled and sieved (flour and grit). The groundnut grain were sorted, toasted, dehulled and milled into paste. The paste was substituted (0- 50%) into the maize flour, cooked and cooled down (32°C). The resulting batter was inoculated with baker's yeast (0.5%) and allowed to ferment (12hrs), diluted with *trona* , salted, stirred vigorously to incorporate air, sized, fried to produce *masa*. The effects of the substituted groundnut paste on physical (thickness, volume, spread ratio), chemical (fat, moisture, ash, protein, carbohydrate) and sensory(colour, texture, taste, odour) qualities on the maize based *masa* were determined. The thickness, loaf volume, specific loaf volume or loaf index and the spread ratio increased from 3.40 to 3.93 cm, 100 to 148cm^3, 1.16 to 1.78 and 3.17 to 3.98, respectively, while the weight decreased from 86.04 to 83.3g with increase in percentage groundnut paste. The protein, fat, carbohydrate and calorie value of the groundnut-maize enriched *masa* increased from 9.56 to 13.59%, 9.48 to 13.23% and 64.62 to 66.98% and 382.04 to 441.53 cal/g, respectively while the ash and the moisture content decreased from 2.1 to 0.8% and 14.24 to 5.54% respectively with increase in groundnut paste (0 - 50%).The average mean scores for the taste, texture and colour increased from 5.53 to 6.47, 5.22 to 6.87 and 5.15 to 6.80, respectively, with increase in the groundnut paste from 0 to 20% and decreased from thence to 6.33, 5.67 and 6.10 correspondingly with further increase of the groundnut paste to 50%.

Key words: Maize grains, groundnut grain, *masa*

Introduction

Masa is a fermented bread-like product, which is round in shape with brown smooth boy and crippling edges, made in Nigeria from millet, maize or rice flour. *Masa* (or waina) is like the India idle in shape and *dosa* in taste (Nkama and Malleshi, 1998) and different from the Mexican '*Masa*' used in tortilla preparation. *Masa* is a very popular staple food consumed by over 80% (by all aged groups) of the Northern Nigeria population of about 47 million (Nkama, 1993). It is also consumed in Niger, Burkina Faso and Mali (Nkama, 1998). *Masa* is prepared to create variety in cereal food products for sale. It is served as breakfast, snack item and sometimes are served with local soup. The brown crisp edges and the mild sour taste are considered by many consumers as the quality attribute required of *masa*. Though *masa* is as popular as Nigeria *ogi,* it receives very little attraction (Nkama and Malleshi, 1998).

A fairly large numbers of research works has been carried out on cereal products and its enrichment (Bacon, 1980; Badi *et al*., 1990; Banigo, 1997; Chavon and Kadam, 1997; Desikachar, 1975; Hofvanda and Underwood, 1997; Hubbel *et al*., 1997; Khetarpul and Chauhan, 1991; Ayo and Olawale, 2003; Ayo *et al*., 2007; Ayo and Gafa 2002) but not much on *masa*.

Masa is consumed in various forms by all aged groups in the Northern states of Nigeria. *Masa* which results from frying of the fermented dough which is round in shape with brown smooth boy and crippling edges. The brown crisp edges and the mild sour taste are considered by many consumers as the quality attribute required of *masa*.

Masa is a good source of income for the producers (*waina*) who prepares the traditional product on sale. The addition of cowpea, groundnut or soybeans flour into *masa* during preparation improved the nutritional quality of *masa* (Nkama and Malleshi, 1998). It serves as a breakfast and snack item. Though *masa* is as popular as Nigeria ogi, it has received very little attention (Okafor, 1983; Nkama and Muller, 1989).

The raw materials and ingredient including millet, rice, salt, sugar, yeast, *trona* or *mkanwa*, vegetable oil are used. The grain particularly pearl millet or maize is dehulled (rice and *acha* are used directly), washed, soaked (12hrs), dried and milled (disc attrition mill). The ground rice/maze/millet is sieved to produce flour and grits. The grits are added to boiling water and cooked to gelatinization and allowed to cool before mixing with raw flour in the ratio of 1:4. The resulting batter inoculated with bakers yeast and its allowed to ferment over night (14-16hours), salt and sugar are added to the inoculums. The fairly thick batter is then diluted with *trona* (*Kanwa* water) an the batter is stirred (vigorously to incorporate air) and fried in a cup-like depression in which oil has been added to produce *masa*.

The problem of *masa* apart from the short shelf keeping quality, is that of low protein content and inconsistence in the use of varied cereals and spices which has resulted in variations in the quality of the product. The work is a follow up of effect of different cereals on the

quality of *masa*, in which maize has been found to compared favourably with rice that has been commonly and long time used for the product. The work is aimed at assessing the effect of groundnut paste on the quality (physical, chemical and sensory) of maize based *masa*.

Materials and Methods

The groundnut seed was cleaned and roasted and grind into flour and added in varying proportion as shown in Table 1 for different formulations.

Maize (*Zea mays* L.) and active bakers yeast (*Saccaronyces cerevesiae*) used for the work were purchased from Jos Central Market, Plateau State. *Kanwa* or *trona* (Sodium bicarbonate) was purchased from Yelwa Market, Bauchi State, Nigeria. The recipe for production of masa is shown in Table 1. The maize grain was cleaned, washed, dry, milled, sieved into grits and flour. The maize grits (1/4 portion) was cooked and cooled down (32°C),mixed with the maize flour (¾ portion) . The resulting batter was inoculated with bakers yeast(1.0%) and allowed to ferment overnight (12hours at room temperature 32°C). The fairly thick batter was then diluted with 10cm^3 *trona solution* (20%). Salt (pitch) and sugar (6%) was added to the batter, stirred vigorously (using a mortar and pestle to incorporate air) and fried (in a local clay pot with individual cuplike depression in which 12cm^3 oil has been added). The batter was fried for 4 minutes on one side, then turned with a small spoon and the other side fried (frying time varies from 6 to 8 minutes) to produce *masa*.

The thickness and width of the *masa* ball was measured using micrometer and ruler, respectively. The loaf volume was determined using seed-displacement method (Ayo, 2003), while the loaf volume was calculated by dividing the loaf volume by the weight of the *masa* (Gomez, 1997).The chemical quality (moisture, fat, protein, ash and carbohydrate) were determined (AOAC, 1990).The calorie was calculated by multiplying the nutrient content by factors 4, 4 and 9 for carbohydrate, protein and fat respectively. The sensory qualities of the *masa* were later subjected to sensory evaluation by 20 untrained panelists (students and staff) from the polytechnic community. Attributes assessed include flavour, taste, colour, texture appearance and the overall acceptability of *masa* using Nine Hedonic scale (1 and 9 for extremely dislike and extremely like, respectively). The data collected were analyzed using ANOVA method (Ihekoronye and Ngoddy, 1985).

Table 1: Recipe for production of groundnut-maize enriched *masa*

	Sample						
Raw Materials	A	B	C	D	E	F	G
Maize /millet	500	500	500	500	500	500	500
Groundnut seed	0	25	50	75	100	125	250
Water	600	600	600	600	600	600	600
Sugar	30	30	30	30	30	30	30
Trona	10	10	10	10	10	10	10
Yeast	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Frying oil	12	12	12	12	12	12	12
Salt	Pinch	Pinch	Pinch	Pinch	Pinch	Pinch	Pinch

Results and Discussion

Effects on Physical Qualities of Maize based *masa*: The effect of groundnut paste on the physical quality of maize based *masa* are summarized in Table 2. The thickness, loaf volume, specific loaf volume or loaf index and the spread ratio increased from 3.40 to 3.93cm, 100 to 148cm^3, 1.16 to 1.78 and 3.17 to 3.98, respectively, while the weight decreased from 86.04 to 83.3g with increase in the percentage of added groundnut paste (0-50%). The increase in the thickness , specific loaf and spread ratio could be due to the increase in the protein and fat content with added groundnut paste. This agreed with the finding of Fennema (2001); Woodroof (1966); Banigo (1997); Ayers and Davenport, 1997; Nkama (1993) that plant protein has high foaming capacity which could give their products an advantage of rising or spreading. Fats, particularly from plant sources has been noted to improve the spreading ratio of their food products commonly in baking and toasting treatment (Woodroof, 1966; Kent, 1984; Ihekoronye and Nggody, 1985; Nkama, 1993). The addition of *trona* (Sodium bicarbonate) has been noted to assist leavening and sponginess of the *masa* (Nkama, 1993). The decrease in the weight of the *masa* with addition of groundnut paste could be due to increase in the oil content in the paste which has been proofed to be relatively lighter.

Effect of groundnut concentrate on the chemical qualities of maize based *masa:* The effect of groundnut paste on the chemical quality of *masa* is summarized in

Table 2: Effect of groundnut on the physical quality of maize *masa*

Maize Grit/ flour%	Ground nut %	Thickness (cm)	Weight (g)	Loaf volume (cm)	Loaf volume index (cm^3/g)	Spread ratio
100	0	3.40±0.8^c	86.04±4.5^c	100±6.2^d	1.16±0.2^c	3.17±0.8^d
95	5	3.48±0.3^{bc}	85.6±4.3^{bc}	110±5.2^{cd}	1.29±0.3^c	3.37±0.4^c
90	10	3.56±.0.8^b	84.0±5.2^{bc}	120±4.3^c	1.43±0.3^c	3.48±0.5^c
85	15	3.58±0.2^b	83.9±2.4^b	132±5.4^b	1.57±0.1^b	3.61±0.5^b
80	20	3.63±0.4^b	83.6±2.1^b	138±8.3^b	1.65±0.4^b	3.78±0.2^b
75	25	3.70±0.4^b	83.5±3.4^b	145±4.6^b	1.73±0.4^b	3.84±0.3^{ab}
50	50	3.74±0.3^a	83.3±3.2^a	148±6.4^a	1.78±0.2^a	3.86±0.4^a

Mean score having the same alphabet along the same column are not significantly different p = 0.05.

Table 3: Effect of ground on the chemical quality of maize *masa*

Maize %	Ground nut %	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Carbohydrate (%)	Calorie (Cal/g)
100	0	9.56±1.2^{c}	9.48±0.6^{c}	2.1±0.2^{a}	14.24±2.1^{a}	64.62±5.3^{a}	382.04
95	5	9.79±0.5^{c}	9.69±0.8^{c}	1.9±0.4^{a}	13.4±1.4^{a}	65.22±6.2^{a}	387.25
90	10	10.06±1.3^{bc}	9.90±0.9^{c}	1.76±0.2^{ab}	11.8±1.3^{b}	66.48±4.2^{a}	395.26
85	15	10.28±0.7^{b}	10.15±1.2^{bc}	1.62±0.5^{b}	10.5±0.9^{b}	67.45±3.2^{a}	402.27
80	20	10.56±1.0^{b}	10.43±1.3^{b}	1.46±0.3^{b}	9.6±0.5^{bc}	67.95±4.2^{a}	407.82
75	25	11.06±1.3^{b}	10.73±0.8^{b}	1.23±0.1^{c}	8.3±0.5^{c}	68.68±5.2^{a}	415.53
50	50	13.59±0.8^{a}	13.23±0.8^{a}	0.8±0.2^{d}	5.4±0.2^{d}	66 .98±2.3a	441.53

Mean score having the same alphabet along the same column are not significantly different p = 0.05.

Table 4: Effect groundnut paste on the sensory quality of maize based *masa*

Maize Grit/flour (%)	Groundnut Paste (%)	Taste	Texture	Colour	Odour	Appearance	General Acceptability
100	0	5.53±1.30^{c}	5.77±1.43^{b}	5.13±1.30^{d}	5.93±1.16^{b}	5.80±1.52^{d}	6.07±1.03^{d}
95	5	5.60±1.59^{c}	5.87±0.99^{b}	5.53±0.99^{c}	5.93±1.16^{b}	6.20±0.91^{c}	6.30±1.35^{c}
90	10	5.93±1.48^{b}	6.20±1.01^{ab}	6.03±0.99^{b}	5.67±1.67^{b}	6.67±0.74^{b}	6.27±1.16^{c}
85	15	6.37±1.22^{a}	6.73±0.81^{a}	6.53±0.91^{a}	6.47±1.30^{ab}	6.97±0.99^{a}	6.73±1.16^{b}
80	20	6.47±1.30^{a}	6.87±1.30^{a}	6.60±1.24^{a}	6.73±1.17^{a}	7.04±0.96^{a}	7.13±1.10^{a}
75	25	6.33±0.72^{a}	6.33±0.61^{a}	6.70±0.56^{a}	6.70±0.75^{a}	7.10±0.67^{a}	6.90±0.67^{a}
50	50	6.02±0.59^{b}	5.67±0.70^{b}	6.10±0.77^{b}	5.87±0.91^{b}	6.30±0.65^{c}	5.98±0.74^{d}

Mean score having the same alphabet along the same column are not significantly different p = 0.05.

Table 3. The protein, fat, carbohydrate and calorie value of the groundnut-maize enriched *masa* increased from 9.56 to 13.59%, 9.48 to 13.23% and 64.62 to 66.98%,and 382.04 to 441.53 cal/g, respectively while the ash and the moisture content decreased from 2.1 to 0.8% and 14.24 to 5.54%, respectively, with increase in groundnut paste (0- 50%). The increasing effect of the groundnut paste on the protein and fat was significant, p =05.The increase could be due to added groundnut paste which has been proofed to contain high quantity of protein(36-40%) and fat(24-36%)(Ayers and Davenport 1997; Nkama 1993; Ihekoronye and Ngoody 1985).
Groundnut-maize enriched *masa* could be a source of protein to the consumer particularly in a developing country like Nigeria where cost of feeding on animal source of protein is unaffordable. The high calorie content of groundnut-maize *masa* could be due to the high fat content of the added paste.

Effect of groundnut concentrate on the sensory qualities of maize based *masa*: The effect of the added groundnut paste to the maize based *masa* is summarized in Table 4.The average mean scores for the taste, texture and colour increased from 5.53 to 6.47, 5.22 to 6.87 and 5.15 to 6.80, respectively, with increase in the groundnut paste from 0 to 20% and decreased from thence to 6.33, 5.67 and 6.10 correspondingly with further increase of the groundnut paste to 50%. The average means score of the odour and appearance increased from 5.80 to 6.7 and 5.80 to 7.10, respectively, with increase in the groundnut paste from 0 to 25% and then decreased to 5.87 and 6.30 correspondingly. The effect of the groundnut paste on the assessed qualities is significant at above 25%, p = 0.05, and is poorly acceptable The general acceptance quality evaluation showed the maximum acceptability of the product at 20% groundnut paste enrichment. The general acceptable improvement on the assessed quality up to 25% could due to combined effects of fermentation by-products (Kordylas, 1990) and interactive effect of groundnut protein and the carbohydrate content of maize flour at the high temperature of frying (Fennema, 2001).

Conclusion: The research has shown that maize based *masa* can be enriched acceptably with respect to sensory quality, up to 25% groundnut paste, with corresponding increase of the nutrient content from 9.56-11.06% (19.87% increase), 9.48-10.73% 13.18% increase), 64.62 to 68.68% 6.28% increase) and 382.04 to 441.53cal/g 15.57% increase) for protein, fat, carbohydrate and calorie, respectively. It could therefore be inferred that the acceptance of groundnut-maize based *masa* by the populace could improve their protein and fat intake.

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Investigation of the Effects of Statin Therapy on Serum Vitamin E Status in Patients with Dyslipidemia

Majid Ghayour-Mobarhan[1,2], Amirhossein Sahebkar[1,3], B.J. Starkey[4,5], C. Livingstone[4,5], T. Wang[4,5], D.J. Lamb[4] and G.A.A. Ferns[4,5]
[1]Heart and Vascular Research Center, Avicenna Research Institute, Mashhad University of Medical Science (MUMS), Mashhad, Iran, Post Code: 9196773117
[2]Department of Nutrition and Biochemistry, Faculty of Medicine, MUMS, Mashhad, Iran, Post Code: 9138813944
[3]Faculty of Pharmacy, MUMS, Mashhad, Iran, Post Code: 91775-1365
[4]Centre for Clinical Science and Measurement, School of Biomedical and Molecular Science, University of Surrey, Guildford, Surrey, England
[5]Department of Clinical Biochemistry, The Royal Surrey County Hospital, Egerton Rd, Guildford, Surrey, England GU2 7XX., England GU2 7XH

Abstract: There are limited data on the effects of statins on serum vitamin E status in dyslipidaemic patients, and no comparisons between statins have been published previously. We have investigated the effect of Atorvastatin and Simvastatin on serum vitamin E status in dyslipidaemic patients. A total of 20 dyslipidaemic patients (14 males, 6 females, mean age 49.15±3.28 years), previously not treated with a lipid lowering agent, were recruited into the study. These patients were randomized to treatment group and received either: Simvastatin 10mg/day (n = 11) or Atorvastatin 10mg/day (n = 9) for 4 months. The control group comprised 14 patients from the same clinic, who were given lifestyle advice, but whose drug treatment remained unchanged for the duration of the study. Serum concentrations of vitamin E, high sensitivity C-reactive protein (hs-CRP) and fasted lipid profiles pre- and post-treatment were measured in all subjects. There were the expected significant reductions in serum lipids in the patients treated with either statin ($P<0.001$). Overall statin treatment was also associated with a significant reduction in serum vitamin E (21%, $P<0.001$) and hs-CRP (45%, $P<0.05$). There was no significant change in these parameters in the control patients. The serum vitamin E / total cholesterol ratio was not significantly altered in patients receiving Atorvastatin, or Simvastatin despite the significant reduction in serum vitamin E. No change in vitamin E status was observed in the controls.

Key words: Atorvastatin, simvastatin, vitamin E, cholesterol, high sensitivity C-reactive protein (hs-CRP)

Introduction

Inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme. A reductase or statins constitute the most powerful class of cholesterol lowering drugs. The use of these agents in the treatment of dyslipidaemia has been shown to improve survival and significantly reduce the onset of cardiac events, in both primary and secondary prevention (Anonymous, 1994; Heart Protection Collaborative Study Group, 2002b; Downs *et al*., 1998; Sacks *et al*., 1996; Shepherd *et al*., 1995).

Over the recent past the so-called pleiotropic actions of statins (effects unrelated to their cholesterol lowering properties) have emerged as being potentially important (Blake and Ridker, 2000; Munford, 2001; Takemoto and Liao, 2001). These properties include anti inflammatory, immunoregulatory and antioxidant effects (Blake and Ridker, 2000; Takemoto and Liao, 2001). Statin-induced reductions in C-Reactive Protein (CRP) provide support for the anti inflammatory effect of these agents (Ridker *et al*., 2001; Ridker *et al*., 1999). Furthermore, it has been proposed that statins improve endothelial function, limit oxidative processes, stabilize atherosclerotic plaques and inhibit the thrombogenic response (McFarlane *et al*., 2002; Node *et al*., 2003).

Vitamin E is a major lipid-soluble antioxidant in cellular membranes. Epidemiological studies indicate that a high intake of dietary vitamin E is associated with decreased CHD risk (Klipstein-Grobusch *et al*., 1999; Knekt *et al*., 1994; Kushi *et al*., 1996; Rimm *et al*., 1993; Smith *et al*., 1996) and this has been proposed to be due to the ability of vitamin E to inhibit cell-mediated LDL oxidation by reducing cellular production and release of reactive oxygen species.

It has been demonstrated that dietary supplementation with vitamin E can inhibit the oxidation of LDL (Princen *et*

Corresponding Author: G.A.A. Ferns, Center for Clinical Science and Measurement, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, UK.

Table 1: Baseline demographic data for groups of patients treated with statin

	n	Mean age (years)	Male/female Sex ratio	Current smoking habit no (%)	Former smoking habit no (%)	Diabetic no (%)	Hypertensive no (%)	CHD No (%)
Atorvastatin	9	45.3 ± 5.5	7/2	0 (0)	4 (44)	2 (22)	4 (44)	0 (0)
Simvastatin	11	52.3 ± 3.9	7/4	2 (18)	3 (27)	2 (18)	3 (27)	0 (0)
Combined	20	49.2 ± 3.3	14/6	2 (10)	7 (35)	4 (20)	7 (35)	0 (0)

Categorical data were compared by Fisher's exact tests. No significant differences were found. The mean ages of the groups were compared by student's t-test, and did not differ significantly.

al., 1992; Reaven, 2002). Not all antioxidants share vitamin E's ability to inhibit the oxidation of LDL. It has also been reported that vitamin E supplementation in the patients with existing CHD may have a beneficial effect in preventing new myocardial infarction (MI) and delaying the progression of arterial damage. It is unclear whether vitamin E is of any benefit in delaying or preventing restenosis in patients who have undergone coronary angioplasty (Hodis *et al.*, 1995; Stephens *et al.*, 1996). It has been suggested that antioxidants including vitamin E may be of value in limiting the oxidative damage to the heart muscle that occurs during ischaemia reperfusion (Stephens *et al.*, 1996).

At least part of the beneficial effect of vitamin E against CHD maybe due to decreased platelet ability to aggregate in humans (Reaven, 2002). Vitamin E may also have a beneficial acute effect on vascular endothelial function (Vogel *et al.*, 1997).

Most of the more recent work in animal models has supported the hypothesis that vitamin E supplementation can prevent or slow the development of atherosclerosis, although the results of early animal experiments were equivocal (Verlangieri and Bush, 1992).

The other potentially protective effects of vitamin E include inhibition of smooth muscle cell proliferation, preservation of endothelial function, inhibition of monocyte-endothelial adhesion and inhibition of platelet aggregation (Diaz *et al.*, 1997; Kaul *et al.*, 2001; Nojiri *et al.*, 2001; Pryor, 2000). Although recent intervention trials do not support a protective role for antioxidant supplementation in high risk patients (Heart Protection Collaborative Study Group, 2002a; Lonn *et al.*, 2005; Marchioli *et al.*, 2002) it is still possible that the antioxidants are of benefit in the early phases of disease. Hence, we wished to investigate the effects of statins, on serum vitamin E status in dyslipidaemic patients.

Materials and Methods

Subjects: Twenty patients with dyslipidaemia who were not originally on a lipid lowering agent were recruited from the lipid clinic at the Royal Surrey County Hospital, Guilford, UK. Fourteen patients from the same clinic but in whom life style advice was the first line of intervention was used as a control group. The medication of these latter patients was unaltered for 4 months and the same parameters were measured as for the statin-treated group.

The characteristics of the statin group are shown in Table 1. All patients and controls were informed of the aims of the study and signed the informed consent form before entering the study which had previously been given approval by the South-West Surrey Ethics Committee. Patients with evidence of established CHD and inflammatory disease were excluded from the study.

Statin treatment: The patients treated with a statin were randomized to two groups: Simvastatin 10mg/day (11 patients) and Atorvastatin 10mg/day (9 patients), each for 4 months. All other medications remained constant for the duration of the study.

Blood sampling: Blood samples were collected between 8.30 and 10.30 a.m. after a 12-h fast by venepuncture of the antecubital vein. Blood was collected into plain Vacutainer tubes (Becton-Dickenson, Cowley, Oxford, UK), allowed to clot and then serum removed.

All chemicals were obtained from Sigma (Sigma Chemical Co, Dorset, UK) unless stated otherwise.

Lipid profiles and blood glucose: A full, fasted lipid profile, comprising total cholesterol, triglycerides, and high density lippoprotein (HDL) cholesterol, was determined for each patient. LDL cholesterol was calculated using the Friedwald equation (Friedewald *et al.*, 1972), except for patients with triglycerides >4.0 mmol/l. Lipid and blood glucose measurements were made by routine enzymatic methods using a Bayer Advia 1650 analyzer (Bayer, Newbury, UK).

High-sensitivity CRP: Serum CRP was determined by PEG enhanced immuno-turbidometry on a Bayer Advia 1650 autoanalyser.

Serum vitamin E assay: Serum vitamin E was determined by HPLC (Ferns *et al.*, 2000). Briefly, internal standard (10mg/mld-tocopherol in isopropyl alcohol) was added to 200mL serum and vortex mixed. Aqueous ammonium sulphate (3.9 M) was added (200mL) and the solution was again vortex mixed. After centrifugation (1000 g for 5 minutes), 50mL of supernatant was used for analysis using a prodigy 50mm ODS2 (50x4.6nm) column (Phenomenex Ltd, Macclesfield, Cheshire, UK) with methanol as mobile phase, and UV-detection at 294 nm.

At a flow rate of 1.0 ml/minute, the retention time for internal standard and vitamin E were 6.6 and 8.7

Table 2: Biochemical parameters in patients treated with statins and control group.

	No	Serum total cholesterol (mmol/l)	Serum triglyceride (mmol/l)	Serum HDL - C (mmol/l)
Pretreatment patients (Simvastatin)	11	7.34±0.31	1.68 (1.45-2.10)	1.39±0.08
Posttreatment patients (Simvastatin)	11	5.20±0.23**	1.62 (1.30-1.81)*	1.36±0.08
Pretreatment patients (Atorvastatin)	9	8.40±0.81	2.12 (1.28-3.23)	1.50±0.10
Posttreatment patients (Atorvastatin)	9	6.97±0.94**	1.74 (1.28-3.10)*	1.44±0.12
Pretreatment patients (combined statins)	20	7.82±0.43	1.93 (1.48-3.00)	1.44±0.06
Posttreatment patients (combined statins)	20	6.00±0.47**	1.65 (1.30-2.02)*	1.40±0.07
Pretreatment controls	14	6.09±0.31	1.69 (0.89-2.99)	1.43±0.06
Posttreatment controls	14	6.15±0.29	1.86 (1.00-2.99)	1.46±0.11

Table 2: continued

	Serum LDL - C (mmol/l)	Serum Hs - CRP (mg/dl)	Serum Vitamin E (µg/ml)	Serum Vitamin E/ total cholesterol
Pretreatment patients (Simvastatin)	5.16±0.30	0.98 (0.19-3.43)	18.06±1.74	2.53±0.21
Posttreatment patients (Simvastatin)	3.15±0.24**	0.67 (0.0-3.12)*	13.75±1.88***	2.68±0.32
Pretreatment patients (Atorvastatin)	6.16±2.26	1.69 (0.70-2.52)	19.12±2.24	2.32±0.22
Posttreatment patients (Atorvastatin)	4.48±2.64**	0.85 (0.43-2.08)*	15.37±1.71***	2.31±0.20
Pretreatment patients (combined statins)	5.61±0.40	1.38 (0.54-3.18)	18.81±1.37	2.46±0.16
Posttreatment patients (combined statins)	3.74±0.45**	0.76 (0.40-2.13)	14.92±1.26***	2.56±0.20
Pretreatment controls	3.83±0.24	0.86 (0.34-2.92)	16.62±1.04	2.63±0.14
Posttreatment controls	3.74±0.28	1.15 (0.63-6.61)	16.85±1.30	2.70±0.26

Values are expressed as mean ± SEM, or median and interquartile range. Comparisons between pre-and post- treatment were assessed by paired t-tests for normally distributed data, or by Mann-whitney test for non-parametric data (*P<0.05, **P<0.01,***P<0.001).

minutes respectively. Vitamin E standard, internal standard and quality control material were obtained from BioRad Laboratories Ltd, Hemel Hempstead, UK. The inter-assay precision was 7.6% and 6.9% for low and high control vitamin E material concentrations respectively.

Statistical analysis: Comparisons between pre- and post-treatment biochemical parameters were assessed by paired *t*-tests for normally distributed data, or by a Mann-Whitney test for non-parametric data. Categorical data were compared using Fisher's exact tests. Values are expressed as mean ± SEM, or median and interquartile range for triglycerides and hs-CRP. A p value of <0.05 was considered significant.

Results

Demographic data: As it is shown in Table 1, their was a high prevalence of type II diabetes, hypertension and smoking habit in the patient groups, which is quite typical for a lipid clinic population. The medication for diabetic and hypertensive patients was unaltered during the study, nor did smoking habit change over this period. There was no significant difference in any of the characteristics between Atorvastatin and Simvastatin groups.

Effect of Statins on the Lipid Profile: Treatment with 10mg/day of Atorvastatin, or Simvastatin caused a 27% and 29% reduction in total cholesterol, respectively (P<0.001, Table 2). Statin therapy was also associated with a modest effect on triglycerides (P<0.05, Table 2). No significant effect was seen on HDL cholesterol. There was no significant change in the lipid profile of patients in the control group (Table 2).

Effect of statins on serum hs-CRP: A reduction in median serum hs-CRP concentration was seen in patients treated with Atorvastatin and Simvastatin. The reductions were 50% and 24%,respectively for each group individually and 45% in the groups combined (P<0.05, Table 2). These changes on basal serum hs-CRP concentrations were similar to previous reports of the effects of statin on hs-CRP levels. Again there was no significant change in the control group (P>0.05, Table 2).

Effect of statins on serum vitamin E status: Both statins reduced serum vitamin E significantly, Atorvastatin by 19.7% (P<0.05) and Simvastatin by 22% (P<0.01), and in a group combined by 21% (P<0.001, Table 2). However, the serum vitamin E /total cholesterol ratio was not altered significantly (P>0.05) by treatment with either statin (Table 2). Serum concentrations of vitamin E, nor the vitamin E / total cholesterol ratio changed significantly in the control patients (Table 2).

Discussion

The effects of statins on the lipid profiles of the patients were similar to those previously reported using the doses of Atorvastatin and Simvastatin as we have (Ferns, 2003; Jialal *et al.*, 2001), indicating that the patients were compliant with their medication over the period of the study.

The effect of the statins on serum hs-CRP concentration was also similar to those reported previously for the doses of drug and duration of treatment used in this study (Ferns, 2003).

To date few studies have investigated the effects of statins on serum vitamin E status, and only one has

compared the effects of Simvastatin to Atorvastatin. Furthermore, previous studies have been inconsistent. Passi and colleagues showed that treatment with Atorvastatin, Simvastatin or Pravastatin was not associated with a significant change in serum vitamin E concentrations even using doses up to 20 mg/d (Passi *et al*., 2003). This is difficult to explain as LDL is the major carrier of vitamin E in blood. Leonhardt *et al*. (1997) have reported that the vitamin E content of LDL was unchanged in patients treated with Fluvastatin (Leonhardt *et al*., 1997) which is in accordance with our finding. Three other studies have also concluded that treatment with simvastatin (Colquhoun *et al*., 2005; Vasankari *et al*., 2004), atorvastatin (Vasankari *et al*., 2004) and pravastatin (Blaha *et al*., 1998) is associated with a significant decrease in plasma vitamin E, which is consistent with our results. But they have also reported a significant increase in the vitamin E / total cholesterol ratio, whereas we have found no significant effect on serum vitamin E / total cholesterol following statin treatment. This may be explained by the differences in dosing regime used, and the duration of the studies.

We have shown that serum vitamin E concentrations are reduced in patients treated with either Simvastatin or Atorvastatin, but that lipid standardized values of vitamin E are unaffected at the doses used. It is possible that the vitamin E/ total cholesterol ratio may be affected by the use of higher doses of statins, and we are currently investigating this. However our data suggest that vitamin E status is not adversely affected in patients on low dose statin therapy.

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Xylooligosaccharide Enriched Yoghurt: Physicochemical and Sensory Evaluation

Semee Mumtaz, Salim - Ur - Rehman, Nuzhat Huma, Amer Jamil[1] and Haq Nawaz[2]
National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan
[1]Department of Chemistry, University of Agriculture, Faisalabad, Pakistan
[2]Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan

Abstract: Enrichment of yoghurt with xylooligosaccharide (XO) at different levels was studied with physicochemical and sensory analysis. Yoghurt prepared by incorporation of XO were compared for these characteristics to the yoghurt containing stabilizer (gelatin, 0.4% w/w) in addition to XO. Moisture contents, pH, acidity and total solids were studied. These attributes were significantly affected by the use of stabilizer and rate of XO incorporation. Use of gelatin produced better results in terms of lowering syneresis and improved appearance, body and texture. Addition of XO upto 3.5% did not influence taste and overall acceptability but higher levels contributed aftertaste.

Key words: Xylooligosaccharide, yoghurt, buffalo milk,

Introduction

Yoghurt is a fermented dairy product which has been used since centuries for being nutritious and tasty. Certain therapeutic properties associated with yoghurt have increased both its production and consumption all over the world. Many health benefits like protection against gastrointestinal upsets, lowering cholesterol, improved lactose digestion, enhanced immune response, better protein, iron and calcium assilmilation are due to live bacteria present in yoghurt (Marona and Pedrigon, 2004).

Many non digestible oligosaccharides (NDO) have been identified to be used in food products as functional ingredients because of proved health claims. Xylooligosaccharides (XO) are newly invented functional food ingredient having various valued physiological properties like maintenance of gastrointestinal health, reduction in cholesterol levels and low cariogenicity. Moreover, these NDO have an acceptable odour, having sweetness that is 30% of sucrose with no offtaste and low calorific value, enables its application in processed foods (Vazques *et al*., 2000). Such NDO have positive effect through increase in growth of bifidobacteria (Gibson and Roberfroid, 1995).

Addition of some NDO (as prebiotic) affected the properties of dairy products by increased syneresis, enhanced permeability and tendency to reduce lower shear stress (Ispen *et al*., 2001).

Augmented dietary status of yoghurt which is one of popular fermented dairy product with acceptable sensory qualities, was the basic objective of the study. The main focus of this work was to produce XO enriched yoghurt for enhanced nutritional properties and acceptable sensory attributes. To confirm this hypothesis, physicochemical and sensory characteristics of XO enriched yoghurt at various storage intervals were determined.

Materials and Methods

Buffalo milk was procured from university dairy farm, University of Agriculture, Faisalabad. Spray dried XO was produced in another experiment by chemical and enzymatic hydrolysis of almond shells. Commercial freeze dried starter culture of *lactobacillus thermophilus* and *lactobacillus bulgaricus* was provided by Chr Hansen Co. (A/S, Hors-, Denmark). Sugar and gelatin were purchased from local market.

Experimental planning and sampling

XO enriched yoghurt preparation:

1: Yoghurt was made from buffalo milk standardized at 3% fat, 9% SNF level.
2: Milk was pasteurized at 95°C for 5 min, homogenized, divided into 9 equal batches and cooled to 45-50°C.
3: All batches were inoculated @ 2.5% v/v culture.
4: Control treatment was prepared without addition of stabilizer or XO.
5: Other eight treatments were prepared by incorporation of XO at the levels 1.5%, 2.5%, 3.5% and 4.5%; among which four without addition of stabilizer (gelatin), four with addition of stabilizer @ 0.5%.
6: The blend was incubated at 45°C±1°C in plastic cups (100 ml) for about 4 hours.
7: Samples were stored at 4°C.

Analysis of physicochemical attributes: Yoghurt samples were tested for fat by Gerber method (British Standard Institution, 1955), in the case of yoghurt this was done by using 11.3g sample of yoghurt in the butyrometer. pH was measured using pH meter (WTW pH-340-A, Germany). Acidity and total solids were determined according to the method described in AOAC (1990).

Table 1: Mean values of the physicochemical attributes of the experimental yoghurts

Treat-ment	pH	Acidity (La)	Total Solids	Syneresis ml/100ml)
T_0	4.36[a]	0.79[d]	14.88[c]	7.53[a]
T_1	4.13[b]	0.81[d]	15.05[bc]	6.00[bc]
T_2	3.93[cd]	0.78[d]	15.22[abc]	6.45[b]
T_3	3.75[de]	0.83[cd]	15.36[ab]	6.58[b]
T_4	3.60[e]	0.93[a]	15.51[a]	6.06[bc]
T_5	4.15[b]	0.83[cd]	15.12[abc]	5.49[c]
T_6	3.96[c]	0.85[bcd]	15.24[abc]	5.58[c]
T_7	3.77[cde]	0.88[abc]	15.39[ab]	5.59[c]
T_8	3.66[e]	0.91[ab]	15.53[a]	5.77[bc]

*Significant difference were obtained between mean values marked with different letter in the same column.

For syneresis, 5ml of yoghurt was centrifuged at 5000 rpm for 20 minutes at 4°C and separated whey was measured after 1 minute. Whey separation amount was expressed as volume of separated whey per 100 ml of yoghurt (Rodarte *et al.*, 1993).

Sensory evaluation: For assessment of overall acceptability of XO enriched yoghurt was done by a panel of judges from among the faculty and research scholars at NIFST, University of Agriculture, Faisalabad. Panel constituted judges who were trained and familiar for yogurt's attributes and showed their willingness. Appearance, body and texture, flavor and over all acceptability were rated on a5 point scale, scoring 5 for excellent, 1 for poor. As recommended by IDF (1987), the attributes of flavor and body and texture were given priority over others by multiplying their scores by 5 and 4 respectively. Total scores were obtained by adding the scores of all attributes. Yoghurt samples were coded with numbers and presented together to panel members in day light. Water was provided for rinsing mouth after each sample.

Statistical analysis: The parameters of this study were designed according to two factor completely randomized design. Effect of XO incorporation in experimental treatments, with or without stabilizer addition was checked by ANOVA. Duncan's Multiple Range Test was used to conclude statistically different groups (Steel *et al.*, 1997).

Results and Discussion

Physicochemical Attributes of XO incorporated Yoghurt

The results of physicochemical properties of eight types of experimental yoghurts are given in Table 1. Significant increase was found in acidity by the addition of XO and also during the storage period at 4±1°C ($P < 0.05$). This has been observed by some researchers that incorporation of non digestible oligosaccharide in food products increased acidity (Voregen, 1998 and Sako *et al.*, 1999). In the yoghurts enriched with XO up to level of 1.5%, 2.5%, 3.5% and 4.5%, acidity continued to increase till the end of storage. The acidity of XO enriched yoghurt was more than that of control treatment without addition of XO (Table 1). In the same way, pH of all treatments decreased constantly in the storage period; because of slightly acidic nature of XO, higher concentration of XO resulted in lowering pH of experimental yoghurts, while addition of stabilizer up to 0.4% did not affect pH significantly (Table 1).

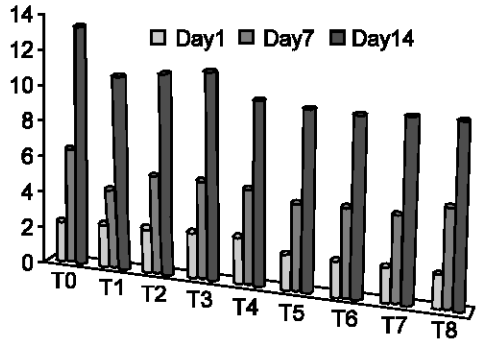

Fig. 1: Syneresis (ml/100ml) study of all experimental yoghurts during storage period.

The total solids of yoghurt increased with the addition of XO and stabilizer while the syneresis declined (Table 1). The results for the effect of storage on syneresis are presented in Fig. 1 which reveals that syneresis increased in all experimental treatments through the entire period of storage. It is obvious from the graph that though addition of XO in yoghurt treatments lowered the syneresis but use of stabilizer up to 0.4% significantly decreased the whey separation. Statistical analysis (Table 1) shows that results were highly significant both for treatments and storage period which are similar to the findings of Lelievre (1977) who examined that the syneresis rate increased with the decrease in pH of yoghurt samples. The increase in syneresis can be due to arrangement of protein network (Walstra *et al.*, 1985). The scores for all the sensory attributes lowered with increased amounts of XO. However, the scores improved with the addition of stabilizer. The yoghurt sample containing up to 3.5% XO with stabilize were found highly acceptable (Fig. 2).

Among sensory attributes, flavor is considered to be the most important factor for determining consumer's response. Results show that effect of storage was highly significant on flavor of all treatments; Kamaruzzaman *et al.* (2002) also reported a decrease in flavor of yoghurt during storage intervals. The addition of XO did not have a bad impact on flavor rather it was quite acceptable (Fig. 2). Texture was affected significantly during storage in all experimental yoghurts; however addition of stabilizer along with XO had a remarkable improvement in scores of experimental yoghurts for body and texture (Fig. 2), which is in accordance with Jawalekar *et al.* (1993) who studied the effect of stabilizer addition on

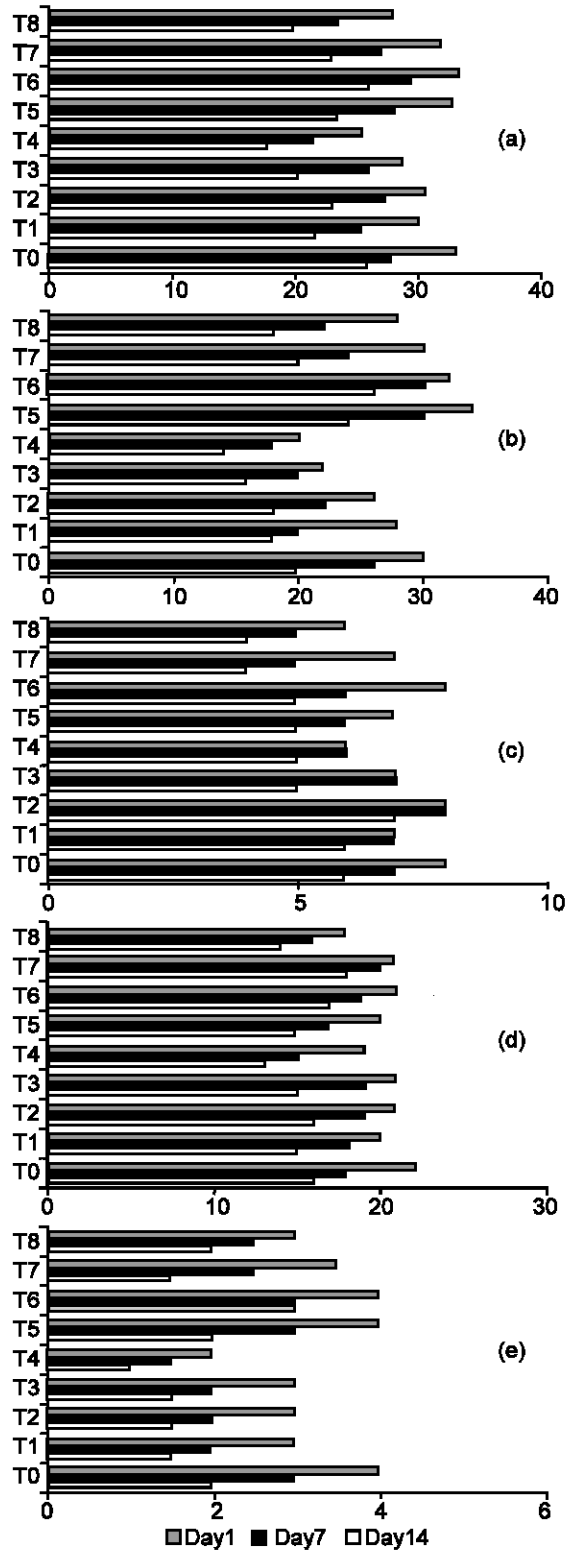

Fig. 2: Sensory evaluation of experimental yoghurts over storage period (a) overall acceptability (b) body and texture (c) aroma (d) flavor (e) appearance.

textural characteristics of buffalo milk yoghurt, concluding increased curd tension, greatest effect by gelatin and least by starch.

Conclusion: The exploring area of functional foods shows considerable promise to expand dairy industry in new arenas. Dairy food fits naturally with both pro- and pre-biotics which have a beneficial effect on the host by altering gastrointestinal flora. Prebiotics are defined as non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of beneficial bacteria in colon. An attempt was made to check the suitability of using XO (NDO/ prebiotic) in yoghurt at different levels with and without stabilizer. It can be concluded keeping the results of organoleptic attributes for all experimental yoghurts that yoghurt enriched with XO up to the level of 3.5% with stabilizer (gelatin, 0.4%) was a successful treatment in terms of overall acceptability, allowing use of XO to have enhanced health benefits associated with this NDO.

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Comparative Studies on Oils from Some Common Plant Seeds in Nigeria

I.E. Akubugwo, G.C. Chinyere and A.E. Ugbogu
Department of Biochemistry, Abia State University, P.M.B. 2000, Uturu, Nigeria

Abstract: Oil were extracted from seeds of Nigerian plants *Pentaclethra macrophylla*, *Treculia africana*, *persea gratesima* and *Telferia occidentalis* using n-hexane and their physicochemical properties compared with oils from seed of *Cocos nucifera*. Percentage oil yield were 20.80, 18.00, 14.00, 18.00 for *P. macrophylla*. *T. africana*, *P. gratesima* and *T. occidentalis* respectively while the value for *C. nucifera* seed oil is 32.00. All the five seed oil were odourless and at room temperature liquids. Specific gravity of the seed oils ranged from 0.81-0.90 while peroxide value for all the oil seed were less than 6.00. Saponification values were as low as 106.60 in *P. gratesima* and as high as 246.00 in *C. nucifera* seed oils. Iodine values were between 9.60 and 52.40 in the extracts. These results suggest that oil seeds examined may be viable source of oil going by their oil yield. The studied characteristics of all oil extracts in most cases compared favourably with C. *nucifera* seed oil which is presently used for both domestic and industrial purposes in Nigeria.

Key words: Oil seeds, physicochemical properties, coconut, conventional seed oils

Introduction

The nutritive and calorific values of seeds make them good sources of edible oils and fats diet (Odoemelam, 2005). Oil seed (groundnut, soyabean, palm kernel, cotton seed, sunflower seed, melon seed olive and locust bean) are the second most valuable commodity in the world trade today (Ige *et al*., 1984).

African oil bean seed (*Pentaclethra macrophylla*) is fermented and consumed. It is consumed alone, mixed with other food ingredients or as a condiment in soup and salads (Achinewhu, 1986). Unfermented seeds are bitter to taste and contain a toxic alkaloid, paucine and a growth depressant, cafeoyl-putrescine (Mbadiwe, 1979). Fermentation renders the seed nutritious and non-toxic (Uzogara *et al*., 1990).

African breadfruit (*Treculia africana*) is commonly roasted, cooked, mashed and consumed either directly as snack food or as flour for use in soup thickening and cake (Fasasi *et al*., 2004). *Treculia africana* seeds are rich in amino acids, minerals and fatty acid (Lawal and Bassir, 1987).

Avocado pear (*Persea gratesima*) Gaerth. F belongs to the family lauraceae. Undocumented ethno-medical sources had it that the seed is used for the treatment of obesity, high blood pressure, heart problems, internal heat and hypertensions (Hutchinson and Dalziel, 1964).

Fluted pumpkin (*Telferia occidentals*) is a creeping vegetable shrub that spread low across the ground with large lobed leaves (Horsfall and Spiff, 2005). The seed contain 13% oil (Okoli and Nyanayo, 1988) and is used for cooking and marmalade manufacturing (Egbekun *et al*., 1998).

Coconut (*cocos nucifera*) is commercially viable. The coconut oil is particularly useful.

Except for *C. nucifera* seed the potential of the other plant seed are presently under-utilized. Our attention has been recently drawn to underutilized plant seeds in Nigeria (Akubugwo and Ugbogu, 2007).

Materials and Methods

Healthy seeds of *P. macrophylla*, *T. africana*, *P. gratesima*, *T. occidentalis* and C. *nucifera* were collected from Isiala Ngwa, Abia State Nigeria between June and November, 2006. They were taken to the department of Biochemistry Abia State University, Uturu. The seeds were authenticated by a taxonomist. They were dehauled (where applicable) sun-dried, wrapped in polyethylene bags and kept in a desiccators until needed.

Extraction of oil: Exactly 300g each of the samples were milled into paste using thermal Willey Mill (model Ed-5), the paste was transferred into a thimble and oil extracted using normal hexane in vacou with soxhlet apparatus. The extracting solvent was evaporated leaving the concentrated oil sample for analysis. Extracted oil was quantified gravimetrically.

Chemicals used: All chemical used were of the analytical grade and products of British drug House Poole England.

Statistical analysis: All extractions and analysis were performed in triplicates. Results were expressed in mean±S.D. statistical significance was established using Analysis of variance (ANOVA). Means were separated according to Duncans multiple range analysis ($p<0.05$).

Experimental: Specific gravity was determined using specific gravity bottle according to the method described by Pearson, 1980.

Iodine value (Wiji's method), saponification number, acid value, peroxide values were as recommended by the AOAC, 1984.

For Iodine value of each sample 0.20g of oil was dissolved in 15 mL carbon tetrachloride in 100 mL glass stoppered flask. 25 mL of Wiji's solution was added, the flask stoppered and allowed to stand for 2 hours in the dark at 25°C 20 mL of 10% potassium iodide (kI) solution was added and mixture titrated with 0.2N sodium thiosulphate ($Na_2 S_2 O_3$) using starch indicator. A blank determination was carried out and the Iodine value calculated using the formula

$$\text{Iodine value} = \frac{12.69N\,(V_2-V_1)}{W}$$

Where N = Normality of thiosulphate
V_1 = Volume (mL) of thiosulphate solution used in test.
V_2 = Volume (in mL) of thiosulphate solution used in blank
W = Weight of sample (0.20g).

Saponification value of the oil samples were determined as described below: 1g of each oil was dissolved in 12.5 mL of 0.5% ethanolic KOH and the mixture refluxed for 30 minutes. 1 mL of phenolphthalein indicator was added and the hot soap solution titrated with 0.5N HCl. A blank determination was also carried out under the same condition and saponification value determined using the equation.

$$\text{Saponification value} = \frac{56.1N\,(V_1-V_2)}{W}$$

Where N = Normality of Hydrochloric acid used
V_1 = Volume of Hydrochloric used in test
V_2 = volume of Hydrochloric acid used in blank
W = Weight of oil used (1g)

For peroxide value (Pv), 1g of each oil sample was weighed into a 200 mL conical flask then 25 mL of 2:1 v/v glacial acetic acid chloroform solvent was added 1 mL of saturated potassium iodine was then added and mixture left in the dark for 1 minute. Next, 30 mL of water was added and the mixture titrated with 0.02N thiosulphate solution using 5 mL starch as indicator. A blank determination was similarly carried out.

Pv was calculated from the equation

$$\text{Peroxide value (PV)} = \frac{\{100\,(v_1-v_2)\ \text{meg/kg}\}}{W}$$

W = weight of sample
V_1 = volume (mL) of thiosulphate used in test
V_2 = volume (mL) of thiosulphate used in blank
N = Normality of thiosulphate ($Na_2S_2O_3$)

Acid value was determined for each oil sample by dissolving 0.20g of each oil in 2.5 mL of 1:1 v/v ethanol: diethylether solvent and titrating with 0.1N sodium hydroxide while swirling using phenolphthalein as indicator. Calculation is as follows

$$\text{Acid value} = \frac{\{56.1\times N\times V\}}{W}$$

Where N = Normality of NaOH used
V = Volume (mL) of NaOH used
W = Weight of sample used

Percentage free fatty acid (%FFA) as oleic) was determined by multiplying the acid value with the factor 0.503. Thus %FFA = 0.503×acid value.

Results and Discussion

The studied physical properties of oil extracts of five Nigerian seeds are shown in Table 1. The n-hexane extractable oil from the four seeds were lower than the 32.00±0.40% obtained for coconut in this study. The percentage oil yield were 20.80±1.20% for *P. macrophylla*, 18.00±1.00% for *T. africana*, 16.00±0.20% for *P. gratesima* and 18.00±2.00% for 1. *occidentalis*. The oil yields for the five studied seeds except (*P. gratesima*) are equal to or higher than 18% reported for soyabean but lower than 43% reported for groundnut seed (Ene-Obong and Carnovale, 1992; Apata and Ologhobo, 1994). They are however, higher than 12.00±0.28 reported for seeds of C. *albidum* an under exploited plant found in Nigeria (Akubugwo and Ugbogu, 2007). The oil yields of all the seeds may be classified as average yielding except for C. *nucifera* seed which is high oil yielding. At room temperature (29°C) all the seed oil are liquids. The Avocado pear oil is Reddish brown while the other seed oils are pale to yellow in colour. The specific gravity of the oils ranged between 0.81 for *T. africana* and 0.90 for *P. gratesima* oil. These values are within the range of specific gravities reported for other fats and waxes (Ajayi and Oderinde, 2002). None of the seed oils had offensive odour.

The chemical properties of the studied seed oils are shown in Table 2. It indicates that the iodine values ranged from 9.60±0.02 in C. *nucifira* to 52.40±2.0 in *P. gratesima*. These values classify the oils as non drying. The relatively low iodine numbers may be indicative of the presence of few unsaturated bond and low susceptibility to oxidative rancidity (Eka, 1980). Acid value is used as an indicator for edibility of oil and suitability for use in the paint industry. The acid value ranged from

Table 1: Physical Properties of oil extracts of five selected Nigerian seed oils

Plant	Percent oil yield	Specific gravity	State at 29°C	Colour	Odour
Pentaclethra macrophylla	20.80^{C}±1.20	0.89$^{a\pm}$0.02	Liquid	Yellow	Agreeable
Treculia africana	18.00^{b}±1.00	0.81^{a}±0.02	Liquid	Yellow	Agreeable
Persea gratesima	16.00^{a}±0. 20	0.90^{a}±0.02	Liquid	Reddish brown	Agreeable
Telferia occidentalis	18.00^{b}±2.00	0.83^{a}±0.02	Liquid	Yellow	Agreeable
Cocos nucifera	32.00^{a}±0.40	0.86^{a}±0.01	Liquid	Pale Yellow	Agreeable

Figures are mean±S.D. Figures bearing different alphabets differ significantly ($p<0.05$): N = 3

Table 2: Chemical Properties of oil extracts of five selected Nigerian seed oils

Plant	Acid value (Meq kg^{-1})	Percentage free fatty acid	Peroxide value	Iodine value	Saponification value
Pentaclethra macrophylla	2.81^{a}±0.01	1.40^{a}±0.01	2.35^{b}±0.41	20.50^{b}±2.0	209.40^{C}±5.0
Treculia africana	8.41^{b}±0.01	4.22^{b}±0.01	1.75^{a}±0.70	27.50^{C}±1.5	212.90^{C}±5.10
Persea gratesima	11.46^{C}±0.16	5.77^{C}±0.07	5.73^{C}±0.22	52.4^{d}±2.0	106.60^{a}±3.60
Telferia occidentalis	3.97^{a}±0.09	1.98^{a}±0.03	2.90^{b}±0.50	49.4^{a}±2.0	158.40^{b}±3.40
Cocos nucifera	11.31^{C}±0.12	4.80^{b}±0.06	0.39^{a}±0.08	9.60^{a}±0.02	246.00^{d}±4.20

Figures are mean±S.D. Figures bearing different alphabets differ significantly ($p<0.05$) according to Duncan's Multiple range analysis N = 3

2.81±0.01 for *P. macrophylla* to 11.46±0.16 for *P. gratesima*. Pearson (1976), reported acid values of 4 for sesame, soybean, sunflower and rape seed and 7 for olive seed oil. Free fatty acid values of less than 3 were obtained for *P. macrophylla* and *T. occidentalis* seed oils are within allowable limits for edible oil while the value for *T. africana*, *P. gratesima* and *C. nucifera* are slightly above (Eckey, 1954). The oils could therefore be used as edible oils.

Peroxide values of less than 6 were obtained in all the seed oils. Peroxide value is used as an indicator of deterioration of oils. Fresh oils have values less than 10 mEqkg. Values between 20 and 40 result to rancid taste (having a disagreeable odour) (Pearson, 1976).

Saponification value is used in checking adulteration. Saponification values were *P. gratesima* 106.60±3.60, *T. occidentalis* 158.40±3.40, *P. macrophylla* 209.40±5.0, *T. africana* 212.90±5.10 and *C. nucifera* 246.00±4.20. The relatively high saponification value recorded for all the seed oils is indicative that they have potential for use in the industry (Amoo *et al.*, 2004).

Conclusion: All the physicochemical properties of the seeds oils studied compared favourably with coconut oil and other conventional seed oils such as groundnut and soyabeans. Their colour and odour are agreeable. The seed oils therefore have potential for development for use as industrial oils.

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Replacement Value of Dusa (Locally Processed Maize Offal) for Maize in the Diets of Pullets and Subsequent Early Laying Characteristics

P.A. Vantsawa[1], S.O. Ogundipe[2], I.I. Dafwang[3] and J.J. Omage[4]
[1]Department of Biological Sciences, Nigerian Defense Academy, Kaduna, Nigeria
[2]National Animal Production Research Institute, Ahmadu Bello University, Zaria, Kaduna, Nigeria
[3]NAERLS, Ahmadu Bello University, Zaria, Kaduna, Nigeria
[4]Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna, Nigeria

Abstract: An experiment was conducted to study the replacement value of dusa (locally processed maize offal) for maize in the diets of pullets (9-20 weeks) and their subsequent early laying performance. Three hundred and seventy eight (378) eight weeks old egg type pullets of approximately equal weights were randomly allocated to seven dietary treatments with three replicates of 18 birds each. The seven dietary treatments composed of rations in which graded levels of dusa replaced maize up to 100% in diet seven. At the end of the experiment average feed consumption was significantly ($p<0.05$) lower for the control and it increased as the level of dusa increased in the diets. The final body weight of pullets was better for treatment four which contained equal proportion of maize and dusa in the diets. The cost (N*kg gain) was significantly ($p<0.05$) higher for the control diet and it decreased as the level of dusa increased in the diets. The subsequent performance of birds revealed that the weight of birds at first egg, 10% and at 50% egg production were better for treatment four. The weight of first egg and ages at first egg were better for the diets with higher level of maize. There were no significant differences ($p>0.05$) in ages at 10%, 50%, peak egg production, weight of eggs at peak production and cost (N*dozen egg) for all the treatments. There was a 39.03% savings in cost of production by using dusa in pullets diets.

Key words: Dusa, pullets, cost (N*kg gain), cost (N*dozen egg)

Introduction

While the consumption of animal products such as meat, egg and fish are a regular part of the meal of an American and European, a Nigerian is much more worried about satisfying hunger and so it is a common knowledge that the meals without any piece of meat are a regular diet for a large proportion of Nigerians (Dafwang, 2006). The food and Agriculture Organization (FAO, 1985) of the United Nations recommended a minimum of 56g of protein intake per person per day. Many Nigerians cannot meet this requirement due to cost of animal products (Fasuyi, 2005).

This high cost of animal products is traced to high cost of feed which accounts for about 70-80% of the total cost of production (Ogundipe, 1987; Kehinde *et al*., 2006). In Nigeria the most popularly incorporated cereal grain in feed formulation is maize where it supplies more than half of the Metabolizable Energy (ME) requirement of poultry (Ravindra and Ravindra, 1988; Durunna *et al*., 2000). However, the high cost of maize due to demand by humans for direct consumption contributes to the high cost of the conventional feed (Vantsawa, 2001; Agbede *et al*., 2002). As a result of this high cost, many poultry farms all over the country are folding up despite encouragement by the government.

For the poultry industry to be sustained, alternative and cheaper energy sources must be sort for. Scientists in the past have tried energy by-products to feed poultry and results obtained by such researchers were quite encouraging. For example, Cresswell and Zainuddin (1980) reported that maize bran can replace maize on a weight for weight basis in broiler diet without any compensation being made for the lower energy content of the bran. Fadugba (1989) showed that industrial maize offal is as good as maize in growers rations. Abound *et al*. (1990) fed maize cob-mix to broilers and found that there was no significant difference ($p>0.05$) in final body weight when compared with the control. Atteh *et al*. (1993) reported that Maize Mill Waste (MMW) could replace all the maize in the diets of pullets without adverse effect on the performance and early lay characteristics. However, Velasco *et al*. (1985) found that maize bran replacement for maize in the diets of 20 weeks old pullet gave a decreasing live weight as the inclusion level increased. They concluded that egg production and feed to gain ratio were best when 10% of the bran was included in the diet. Vantsawa *et al*. (2007) reported that dusa can replace all the maize in the diets of chicks without any adverse effect on performance.

This by-product has not been tried as replacement for maize in the diets of pullets. It is available all year round especially in the Northern part of the country where majority of households consume maize flour in form of "tuwo". Dusa production is directly proportional to maize

Table 1: Composition of Experimental Grower Mash

Ingredient/treatment	1	2	3	4	5	6	7
Maize	49.26	40.34	31.43	22.51	13.59	4.67	0.00
Dusa	0.00	10.00	20.00	30.00	40.00	50.00	55.23
GNC	24.49	23.41	22.32	21.24	20.16	19.08	18.52
PKC	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Rice Offal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Blood meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	2.70	2.70	2.70	2.70	2.70	2.70	2.70
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix/TM*	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis							
ME Kcal/kg	2893	2821	2750	2678	2607	2536	2500
Crude Protein (CP)	19.00	19.00	19.00	19.00	19.00	19.00	19.00
Crude Fibre (CF)	4.59	4.66	4.73	4.80	4.81	4.94	4.97
Phosphorus (%)	0.87	0.89	0.92	0.95	0.97	0.99	1.00
Calcium (%)	1.46	1.46	1.46	1.46	1.46	1.46	1.46
Lysine (%)	0.87	0.81	0.81	0.81	0.81	0.80	0.80
Methionine (%)	0.25	0.25	0.25	0.26	0.26	0.26	0.26
Methionine+cystine	0.65	0.61	0.62	0.63	0.64	0.64	0.65
Cost/kg feed (N)	24.22	22.94	21.73	20.50	19.26	18.02	17.37

*Biomix grower premix supplied the following per kg diet: - Vit. A, 10,000 i.u.; Vit D3, 2000i.u; Vit E, 23mg; Niacin 27.5mg, Vit B_1, 1.8mg; VitB_2, 5mg; Vit B_6, 3mg; Vit B_{12}, 0.015mg, Vit K, 2mg; Pantothenic acid, 7.5mg, Folic acid, 0.75mg; Choline choloride, 300mg; cobalt, 0.2 mg, copper, 3 mg; Iodine, 1mg, Iron, 20mg; Manganese, 40mg: Selenium, 0.2mg

Table 2: Composition of Common Layers Mash

Ingredient	%
Maize	20.00
Dusa	35.73
GNC	23.72
PKC	10.00
Limestone	7.00
Bone Meal	3.00
Salt	0.30
Premix/TM*	0.25
Total	100.00
Calculated analysis	
M.E. Kcal/kg	2492
Protein (%)	18.00
P%	0.68
Ca %	3.84
Methionine (5)	0.23
Lysine (%)	0.71
Cystine (%)	0.46
Methionine+Cystine	0.69
Cost/Kg Feed	21.85

•Biomix layer premix supplied the following per kg diet:- Vit. A, 10,000 i.u.; Vit. D3, 2000i.u; Vit. E, 23mg; Niacin 27.5mg, Vit. B1, 1.8mg; Vit. B2, 5mg; Vit. B6, 3mg; Vit. B12, 0.015mg, Vit. K, 2mg; Pantothentic acid, 7.5mg; Folic acid, 0.75mg; Choline chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1mg, Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg

consumption. To get the dusa maize grain is passed through a dehulling machine with a small amount of water added so as to soak the testa. The machine when on, revolves at a very high speed. The pistol rubs off the testa of the grain and thereafter the endosperm which is used for human consumption comes out in one direction while the testa, bran, germ and reasonable quantity of broken endosperm is ejected in the opposite direction. It is the testa, bran, germ and the reasonable quantity of endosperm that constitute dusa. It is about 25% of the total maize dehulled (Vantsawa, 2001). This study was therefore conducted to evaluate the replacement value of dusa for maize in the diets of growing pullets. (9-20 weeks) and its effect on subsequent early lay performance of birds.

Materials and Methods

Three hundred and seventy eight (378) eight weeks old birds of approximately equal weight were randomly allocated to seven dietary treatments with three replicates of 18 birds each. The seven dietary treatments composed of rations in which graded levels of dusa replaced maize up to 100% in diet seven. The birds were reared in an open-sided deep litter house screened with wire mesh. The growers mash was formulated as shown in Table 1.

Feed and water were given *ad-libitum* and Mortality was recorded as it occurs. Feed consumption and weight gain were recorded every two weeks. Vaccination and other management practices like debeaking were done on schedule. Cost (N*kg) Feed cost (N*kg gain) and cost (N*kg bird) were computed for the grower phase.

After the final weights of birds were taken at week 20, the birds were switched over to a common layers mash as shown in Table 2. Feed and water were provided *ad-libitum*. Record of mortality was taken as it occurs. Other records taken include body weight at first egg, body weight at 10%, 50%, peak egg production and cost per dozen eggs. All the data collected were subject to the analysis of variance using the general linear statistical model (SAS, 1990).

Trend analyses were done for average daily feed consumption, average weight gain, feed to gain ration, cost (N*kg gain) and cost (N*kg bird). Differences

Table 3: Effect of Graded levels of Dusa as a Replacement for Maize on the Performance of Grower (9-20 Weeks)

Levels of Dusa	0.00	10.00	20.00	30.00	40.00	50.00	60.22		
% Maize Replaced	0.00	16.60	33.22	49.86	66.47	83.07	100.00		
Parameters/Treatment	1	2	3	4	5	6	7	SEM	LQ
Avg total feed cons. (g)	5406.84^{e}	5481.20^{dc}	5558.82^{d}	5786.38^{c}	5831.47^{bc}	5960.74^{ab}	5997.22^{a}	46.13	*
Avg final wt of birds (g)	1389.67^{c}	1383.33^{c}	1382.00^{c}	1445.00^{a}	1424.00^{ab}	1411.00^{bc}	1415.00^{bc}	11.65	*
Average wt gain (g)	778.56^{b}	771.76^{b}	772.74^{b}	832.04^{a}	812.43^{ab}	801.00^{ab}	805.74^{ab}	12.62	*
Feed to gain ration	6.95^{a}	7.11^{a}	7.20^{ab}	6.94^{a}	7.18^{ab}	7.44^{b}	7.45^{b}	0.10	*
Cost (N/kg gain)	154.20^{a}	150.02^{a}	141.16^{b}	126.35^{c}	120.11^{d}	113.53^{e}	02.51^{f}	2.04	*
Cost (N/ bird)	214.29^{a}	207.53^{a}	195.08^{b}	182.58^{c}	171.04^{d}	160.23^{e}	145.05^{f}	2.00	*

Means along the same row bearing the same superscript are not significantly difference (p>0.05); SEM = Standard error of the means; L,Q = Polynomial showing linear and quadratic relationship across the treatments

Table 4: Effect of Graded Levels of Dusa on Subsequent early laying Performance of Pullets 20-32 Weeks

Levels of Dusa	0.00	10.00	20.00	30.00	40.00	50.00	60.22		
% Maize Replaced	0.00	16.60	33.22	49.86	66.47	83.07	100.00		
Parameters	1	2	3	4	5	6	7	SEM	Q
Body wt at first egg (g)	1428.00^{bc}	1447.00^{abc}	1421.33^{c}	1487.67^{a}	1462.00^{abc}	1466.67^{ab}	1439.67^{bc}	12.66	*
Body wt at 10% Prod (g)	1565.00^{b}	1628.00^{ab}	1573.33^{b}	1685.00^{a}	1620.00^{a}	1641.00^{ab}	1596.33^{b}	24.65	*
Body wt at 50% prod (g)	1666.67^{c}	1693.33^{bc}	1660.00^{c}	1770.00^{a}	1740.00^{ab}	1750.00^{ab}	1676.67^{c}	19.88	*
Age at first egg (days)	147.33^{a}	162.00^{b}	160.00^{ab}	156.00^{ab}	154.33^{ab}	152.67^{ab}	158.00^{ab}	4.14	
Age at 10% prod (days)	159.00	162.00	164.67	164.33	161.00	155.67	158.00	3.28	
Age 50% prod (days)	1 77.33	175.67	176.00	174.33	174.00	171.00	1 75.0	1.91	
Age at peak prod (days)	211.00	215.33	222.33	209.67	196.33	203.00	221.33	9.22	
Weight of first egg (g)	40.83^{b}	50.00^{a}	48.87^{a}	46.70^{ab}	44.37^{ab}	47.53^{ab}	46.67^{ab}	2.89	
Weight of egg at peak (g)	60.78	61.02	59.90	61.87	61.49	61.74	61.46	0.78	
Feed Cost/dozen egg (N)	50.61	51.06	51.26	50.32	51.38	50.75	57.41	0.98	
%hen-day prod.20-32wks	77.06^{ab}	72.68^{b}	73.29^{b}	78.27^{ab}	78.68^{ab}	82.28^{a}	76.04^{ab}	2.28	
%hen-housed20-32wks	76.62^{ab}	72.68^{b}	71.82^{b}	76.04^{ab}	78.68^{ab}	82.26^{a}	76.04^{ab}	2.11	
% Mortality	5.56	0.00	5.56	5.56	0.00	0.00	0.00	0.79	

Means along the same raw bearing the same superscripts are not significantly different; SEM = Standard error of the means;
Q = polynomial showing quadratic relationship across the treatments

between treatment means were separated using Duncan's Multiple Range Test (Steel and Torrie, 1980).

Results

The results of the effect of graded levels of dusa as replacement for maize on the performance of pullets (9-20 weeks) and the subsequent early lay characteristics are presented in Table 3 and 4 respectively. Feed consumption increased linearly from control diet to diet seven (treatment in which all the maize was replaced with dusa). While highest final weight was recorded for birds in treatment four in which there was equal proportion of maize and dusa, the least gain was observed in birds on treatment two in which 10% dusa replaced about 16.6% maize in the diet. The best feed to gain ration was obtained for birds on treatment four. The poorest feed to gain ration was observed for the birds on treatment seven. There was no mortality during this phase of study. The cost (N*kg gain) and cost (N*kg bird) decreased significant ($p<0.05$) as the level of dusa increased in the diet.

The subsequent performance showed that weight of birds at first egg was better for treatment four. This was followed by treatment 6 in which dusa replaced maize at 83.07%. There was no significant ($p>0.05$) difference between treatment five and two and between seven and the control. The least weight was observed in birds on treatment three replacing 33.22% maize. Treatment four had a significantly ($p<0.05$) higher body weight at 10% and 50% egg production.

Treatment 5 and 6 had similar values and both had higher gain when compared to the control treatment. The birds on the control diet came to lay earlier than those on other treatment diets. There was no difference in age at first egg between treatments 3, 4, 6 and 7. Birds on treatment 2 however, had a delayed on set of lay. The weight of egg at peak production did not show any significant difference ($p>0.05$). Similarly no difference was observed in cost (N*dozen) egg. Percent hen day and percent hen-housed were better for treatment 6 and the least were in treatments 2 and 3. There was no significant difference ($p>0.05$) in the percent mortality.

Discussion

The increase in consumption as the level of dusa increased in the diet is in agreement with the work of Fadugba (1989) who observed that feed intake increased for all the industrial maize offal diets than the control. This is also in agreement with the work of Farrel and Johnson (1973) and Olomu (1984) where all reported that feed intake of chickens is inversely related to the dietary energy concentration. Since dusa is lower in energy than the maize (2784Kcal*kg versus 3432 Kcal*kg respectively), that explains why there was a higher feed consumption by birds in treatment seven than all other treatments. Sunde (1984) also observed that feed consumption decreased when the energy contents of the diets increased. The better feed to gain ratio observed for the control diet when compared with other treatments may be as explained by Duarak and

Bray (1978) who observed that when high fibre diets are fed to monogastric animals, there were reductions in digestibility and utilization resulting in poor feed conversion.

The fact that dusa is higher in fibre than maize explains why feed conversion became poorer as the level of dusa increased in the diets. However, despite the poor feed to gain ration observed in treatment 7, the overall advantage in terms of cost saving in using dusa was better than using maize in pullets diets. Dusa can therefore be used in the diets of pullets without any adverse effect on performance with a 39.03% saving in cost of production.

In the subsequent early lay characteristics, the significant difference ($p<0.05$) observed for body weight of pullets at first egg, at 10% and 50% egg production may indicate that dietary levels of dusa did not affect the performance of birds during the growing phase. Atteh *et al*. (1993) observed that Maize Mill Waste (MMW) could replace all the maize in the diet of pullet without any adverse effect on the performance and on early lay characteristics. This is however, contrary to the observation made by Velasco *et al*. (1985) where they reported that performance of laying birds were best at 10% maize bran inclusion in the diets of birds. The differences observed in the weight of first egg may be as a result of age of birds before first lay. The earlier the birds come to lay the smaller the size of the first egg laid. That was why the size of egg in the control was smaller than others. The non-significant difference in egg weight at peak egg production and the higher percent hen day and hen housed egg production for the treatments signifies that high level of dusa can sustain egg production without any significant effect on the performance of birds.

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Effect of Zinc from Zinc Sulfate on Ewes' Weight, Milk Yield, Zn Concentrations in Serum and Serum Alkaline Phosphates Activity of Varamini Ewes

A. Zali, A. Nik-Khah, A. Zare Shahneh, K. Rezayazdi and M. Ganjkhanlou
Department of Animal Science, Faculty of Agronomy and Animal Science,
University of Tehran, P.O. Box # 3158711167-4111, Karaj, Iran

Abstract: This experiment was conducted to investigate the effect of feeding supplemental zinc (zinc sulfate) in different levels (0, 15 and 30 mg/kg) on ewes weight, milk production, Zn concentrations in serum and serum alkaline phosphates activity. Thirty lactating Varaminni ewes were assigned to three experimental groups according to their live body weights, milk production and lambs sex in a completely randomized design. Ewes were fed a basal diet containing alfalfa, wheat straw, cottonseed meal, barley grain, wheat bran, cracked corn and vitamin-mineral supplements at 3.2% of BW to meet NRC requirements for protein, energy, macro minerals and micro minerals. The basal diet contained 15 mg/kg Zn and Zinc sulfate was added to the basal diet to supply 30 or 45 mg/kg of dietary zinc. Milk yielded, milk composition and ewes' weight was recorded at 7 and 21 days intervals respectively. Samples of the blood were taken three times (0, 35 and 64) for determination of Concentration of Zn, Cu and Fe, Na, K, Ca in serum. Also serum alkaline phosphates concentration of ewes was measured. Milk yield, milk composition and ewes' weight of ewes were not affected by supplemental zinc ($p>0.05$). Alkaline phosphatase concentration was increased with supplemental zinc linearly and this increase was significant ($p<0.05$). Blood mineral concentration was not affected by treatment ($p>0.05$).

Key words: Supplemental zinc, zinc sulfate, varaminni ewes

Introduction

Zinc deficiency was reported in many parts of Iranian soils and this phenomenon affected on zinc content of all plant and animal species (Church, 1980). Zinc is a trace element essential for every form of life (Underwood, 1956). Poor growth is a prominent characteristic of Zn deficiency of animal and plant species (Dijkhuizen *et al*., 2001). Zinc is involved in many metabolic processes and, consequently, zinc deficiency leads to a wide range of manifestations, including decreased growth, impaired immune competence and develop mental delay (Allen, 1994; Gibson, 1994). Zinc is essential trace mineral, whose requirements, form (chelate, complex, sulfate, oxide, etc.) and interactions with each other are not clearly understood. Impaired growth is one of the most consistent signs of malnutrition and although it is in itself not hazardous to health, it is associated with poverty, poor health and low production (Hatfield *et al*., 2001).

Hatfield *et al*. (1992) demonstrated the benefit of feeding Zn at six times the NRC (1985) recommended level to feedlot lambs in a stressful environment. High levels of supplemental Zn in the form of Zn were also shown to have a positive influence on ewe milk production and ultimately lamb weaning weights (Hatfield *et al*., 1995). In addition, sheep producers often feed supplemental Zn at concentrations higher than NRC (1985) recommendations to prevent foot infections in sheep subject to damp, muddy, con-finement situations. However, the influence of form of supplemental Zn (organic vs inorganic) on sheep Zn status around NRC recommendation has not been well documented (Rojas *et al*., 1995). Blood plasma serves as an immediate source of stored Zn. However, The objectives of this study were conducted to compare three levels of inorganic Zn (zinc sulfate) around of NRC (1985) recommendation in Iranian sheep by evaluating Zn concentrations in serum, also alkaline phosphates activity and milk production of varamini ewes breed.

Materials and Methods

Animals and location of experiment: Thirty lactating Varaminni ewes (n = 30; initial BW 47.95±2.86 kg) were used in a 70-d study (February 22 to April 30) at the sheep facility of the Animal Science Department of Tehran University in Karaj, approximately 45 km west of Tehran city to determine the effects of supplemental Zn (0 mg, 15 mg and 30mg Zn_ewe-1_d-1) on ewes weight, milk production, Zn concentrations in serum and serum alkaline phosphates activity. Zinc supplements were administered daily for 70 d. Supplies of premixes needed for the trial were prepared prior to the start of the trial by zinc sulfate. The rations were fed to ewes as Total Mixed Rations (TMR), but zinc premix was top-dressed on the a.m. Based on NRC (1985) estimated DMI for a 50-kg lactating ewe of 1.6 kg DMI_ewe-1_ d-1(3.2% BW). The diets were fed twice daily (0800 and, 1600 h) and feed offered was adjusted daily about 5%

Table 1: Components and chemical composition of basal diet

Item	Diet	Water
CP (%)	12.2	---
Zn ppm	15.0	<001
Fe ppm	88.0	<001
Cu	9.0	<001
Ca(%)	0.9	---
P (%)	0.6	---

All of composition on DM basis

orts. The TMR was comprised of 65% forage and 35% of a concentrate mix to formulate diets to meet NRC (1985). diet plus zinc supplements provided 15, 30 and 45 mg/kg DM of Zn, which was approximately around the Zn recommended levels of NRC (1985).

Ewes were managed as one group with ad libitum access to feed, water and white salt and the mineral composition of basal diet and water was presented in Table 1. Ewes were weighed on d 1, 21, 42, 63 and 70 after an overnight shrink without food or water.

Milk yielded of ewes was recorded at 7 days intervals and this value was assumption mean yield for one weak. Samples of the milk were taken once per week for determination of milk composition and milk fat, protein and lactose were determined in centre laboratory in animal science department.

To determine the effect of treatments on blood Zn. on d 1, 35 and 64, blood was collected from each ewe via jugular venipuncture using unheparinized vacutainers (10 mL). Blood was allowed to coagulate at room temperature. Samples were centrifuged for 20 min at 1,000×g, separating blood serum from red blood cells. Serum was decanted into plastic serum tubes, which were then capped and stored frozen at-20°C until analyzed. Samples collected on d 64 were analyzed for alkaline phosphatase activity in Tehran Veterinary Laboratory. Zn, Cu, Fe, Na, K and Ca concentration was determined by flame atomic absorption in kavosh laboratory (AOAC, 1990). Serum alkaline phosphatase activity was also measured as an indication of Zn status using an alkaline phosphatase kit (Sigma Diagnostics, St. Louis, MO).

Experimental design: Ewe was the experimental unit in a completely randomized design that assigned to three experimental groups according to their live body weights, milk production and lambs sex. The procedure of GLM (SAS, 1998) was used to evaluate blood Zn, Fe and Cu status and alkaline phosphatase activity. Also ewes BW and milk production were evaluated using the PROC GLM procedure of SAS (1998).

Results

Ewe BW at the end of the study and ewe BW change from beginning to end of the study did not differ ($p>0.05$) between ewes supplemented with sulfate zinc and control ewes (Table 2). Supplemented ewes with Zn sulfate had no effect ($p>0.05$) on milk production (Table 2). blood Zn concentration was higher in Zn supplemented than control ewes although this increase was not significant ($p>0.05$). Blood Cu, Fe, Na, K and Ca concentration did not differ ($p>0.05$) between ewes supplemented with sulfate zinc and control ewes (Table 3). Serum alkaline phosphatase activity was differ ($p<0.05$) between control and supplemented ewes (Table 4) and alkaline phosphatase activity tended to be greater in supplemented ewes than control group.

Discussion

In the present study, zinc supplement did not affect final BW, BW change and milk production. This agrees with other researcher (Hatfield *et al*., 2001), but Hatfield *et al.* (1995, 1992) in two separated studies, observed zinc supplement in above NRC (1985) recommendation increased feedlot lamb performance and had a positive influence on ewe milk production and, ultimately, lambs weaning weight. Similar results have been reported previously when dairy cows were supplemented with chelated minerals (Formigoni *et al*., 1993). Also milk components were not affected by treatment, which is similar to results in another study (Hansen *et al*., 1994). There were no effects of the Zn supplement on blood plasma element concentrations. Although Serum Zn concentrations increased with supplemental Zn but this increase no significant. Therefore, it is impossible to speculate from these data about tissue Zn retention by the different treatment groups. Element accumulation occurs if homeostatic or homeorhetic mechanisms cannot maintain a constant concentration in the body. The absorption of many essential metals is controlled by these mechanisms, such as Zn, whose absorption can vary from less than 10 to over 80% depending on the animal's status (Underwood and Suttle, 1999). Other mineral concentration in blood unaffected by zinc supplement. In many studies zinc supplement affected tissue concentration of mineral but unaffected on serum mineral concentration (Schell *et al*., 1996). In our study diet had a 15 mg/kg of Zn that this is lower than NRC (1985) recommended.

Serum alkaline phosphatase was increased with Zn supplement and this increase was linearly. This result was supported by other research (Wan *et al*., 1993., Kraus *et al*., 1997) Blood alkaline phosphatase has been used as an indication of animal Zn status. Wan *et al*. (1993) and Kraus *et al*. (1997) reported higher plasma alkaline phosphatase concentrations in Zn-adequate than in Zn-deficient rats. Healy and Davis (1975) reported that total serum alkaline phosphatase activity increased more in lambs fed 100% wheat or 67% wheat-33% alfalfa than in lambs fed diets containing 33% wheat and 67% alfalfa or diets composed of 100%

Table 2: Effect of zinc supplement on final BW and average BW change during 70-d study (Mean±SE)

Items	Control group* (Mean±SE)	Supplemental Zn (15mg/kg)	Supplemental Zn (30mg/kg)	Significance
Final BW (kg)	48.14±4.01	49.00±3.79	48.45±4.02	NS
BW change (kg)	1.30±0.39	1.48±0.42	1.39±0.37	NS

*no supplemental Zn

Table 3: Effect of dietary supplementation with zinc on milk production and composition

	Supplemental zinc (mg/kg)			
	0	15	30	Significance
Milk production[1]	49.30±7.27	49.70±7.53	50.31±7.32	NS
Milk Composition**				
Fat%	4.14±0.93	5.42±0.48	4.78±0.72	NS
Protein%	5.42±0.80	5.98±0.52	5.21±0.85	NS
Lactose%	5.53±0.45	5.48±0.34	5.22±0.42	NS

[1]Milk yield was reported in 70-d

Table 4: Effect of zinc supplement on mineral and alkaline phosphatas concentration in blood (Mean±SE)

Items	Control group	Supplemental Zn (15mg/kg)	Supplemental Zn (30mg/kg)	Significance
Serum Zn (µg/dl)	85±14	91±11	100±10	NS*
Serum Cu (µg/dl)	78±8	78±10	65±7	NS
Serum Fe (µg/dl)	132±11	110±11	99±12	NS
Serum Na (MEq/l)	157±15	130±15	119±16	NS
Serum K (MEq/l)	4.95±0.27	4.8±0.17	4.5±0.29	NS
Serum Ca (Mg/dl)	10.1±1.28	9.4±1.35	9.3±1.51	NS
Serum ALP (U/l)	103±0.001[b]	113±0.008[ab]	131±0.02[a]	0.05

*NS: p>0.05

alfalfa hay. These results reflect the Zn concentrations of wheat and alfalfa. In dogs, alkaline phosphatase did not respond to level or form of Zn supplementation in a consistent manner (Lowe and Wiseman, 1998).

Implication: Feeding supplemental Zn was increased Serum alkaline phosphatase concentration And assumption had good effect on production and sheep health if feeding high level from NRC (1985) recommendation. We recommend this study investigate in pregnancy period and those effected on milk production and ewes and lambs health be study. In region such as Iran that animal species was toiled from Zn deficiency, we recommend Zn was supplemented to diet.

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Comparison of Phenolic Compounds of Some Edible Plants of Iran and India

Ali Aberoumand[1] and S.S. Deokule[2]
[1]Department of Food Science, Ahvaz University, Ahvaz, Iran
[2]Department of Botany, Pune University, Pune, India

Abstract: Phenolic compounds, ubiquitous in plants are an essential part of the human diet and are of considerable interest due to their antioxidant properties. The antioxidant activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings. *Alocacia indica sch.*, *Asparagus officinalis DC.*, *Chlorophytum comosum Linn.*, *Cordia Myxa Roxb.*, *Eulophia Ochreata Lindl.*, *Momordica dioicia Roxb.*, *Portulaca oleracia Linn. and Solanum indicum Linn.* are the major sources of phenolic compounds in the human diet. Soluble phenolic acids were extracted with methanol. The aim of this study determination of the distribution and total phenolic compound in a wide range of vegetables consumed in India and Iran.

Key words: Phenolic, antioxidant, methanol and *solanum indicum linn*

Introduction

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and Phenylpropanoid pathways in plants (Randhir *et al.*, 2004).

These compounds one of the most widely occurring groups of phytochemicals are of considerable physiological and morphological importance in plants. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia, 1997; Manach and Mazur, 2005; Middleton, 2000; Puupponen-Pimia, 2001; Samman, 1998).

Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables (Hertog and Feskens, 1993) and (Parr and Bolwell, 2000). The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity (Heim *et al.*, 2002). Phenolic compounds could be a major determinant of antioxidant potentials of foods (Parr and Bolwell, 2000), and could therefore be a natural source of antioxidants. This review aims to examine the chemistry of phenolic compounds in relation to their antioxidant activity, the occurrence of phenolic compounds in various food and non-food sources, the bioavailability and metabolism of phenolic compounds and also explore the potential use of these compounds as food antioxidants.

Plant polyphenols are known to have multi functional properties such as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers and flavonoids and their derivatives are the largest and most important group of polyphenols. The most important property is their capacity to act as antioxidants protecting the body against reactive oxygen species and may have an additive effect to the endogenous (Shahidi and Naczk, 1995).

Among the variety of phenolic compounds, phenolic acids have attracted considerable interest in the past few years due to their many potential health benefits. As polyphenols, phenolic acids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anti-carcinogenic, anti-inflammatory and vasodilatory actions (Duthie, 2000; Breinholt, 1999; Shahidi and Naczk, 1995).

Food composition databases need to be established to assist in these investigations. The data compiled by (Radtke, 1998; Clifford, 1999) indicate that many vegetables are either moderate or good sources of phenolic acids. However, up-to-date research data on the contents of phenolic acids in vegetables are limited.

The chemistry of phenolic compounds: Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents and range from simple phenolic molecules to highly polymerised compounds (Bravo, 1998). Despite this structural diversity, the groups of compounds are often referred to as 'polyphenols'. Most naturally occurring phenolic compounds are present as conjugates with mono- and polysaccharides, linked to one or more of the phenolic groups and may also occur as functional derivatives such as esters and methyl esters (Harborne, 1989; Harborne, 1999; Shahidi and Naczk, 1995). Though such structural diversity results in the wide range of phenolic compounds that occur in nature, phenolic compounds can basically be categorized into several classes (Harborne, 1989; Harborne, 1999). Of these, phenolic acids, flavonoids and tannins are regarded as the main dietary phenolic compounds (King and Young, 1999).

Structure - activity relationships: The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations (Afanas'ev, 1989 and Amarowicz, 2004). The structure of phenolic compounds is a key determinant of their radical scavenging and metal chelating activity and this is referred to as structure-activity relationships (SAR). In the case of phenolic acids for example, the antioxidant activity depends on the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group (Rice-Evans, 1996) and (Robards, 1999). Monohydroxy benzoic acids with the -OH moiety at the *ortho*- or *para*-position to the -COOH show no antioxidant activity, though the same is not true for the m-hydroxybenzoic acid (Rice-Evans, 1996). The antioxidant activity of phenolic acids increase with increasing degree of hydroxylation, as is the case of the trihydroxylated gallic acid, which shows a high antioxidant activity. However, substitution of the hydroxyl groups at the 3- and 5-position with methoxyl groups as in syringic acid reduces the activity (Rice-Evans, 1996).

Food sources of phenolic compounds: Though phenolic compounds are present in almost all foods of plant origin, fruits, vegetables and beverages are the major sources of these compounds in the human diet (Hertog *et al*., 1993).

Fruits and vegetables: There are wide variations between the total phenolics contents of the different fruits or vegetables, or even for the same fruits or vegetables reported by different authors these differences may be due to the complexity of these groups of compounds and the methods of extraction and analysis (Bravo, 1998; Kalt, 2001). For example, phenolic compounds present in fruits are found in both free and bound forms (mainly as β-glycosides), but as the latter are often excluded from analyses, the total phenolics contents of fruits are often underestimated (Sun *et al*., 2002). Besides, phenolics contents of plant foods depend on a number of intrinsic (genus, species, cultivars) and extrinsic (agronomic, environmental, handling and storage) factors (Tomas-Barberan and Espin, 2001; Rapisarda, 1999). Species differences are also pronounced, as observed from the data in Table 2, which suggests that the phenolics content of some fruits, i.e., banana, litchi (lichee), mango and persimmon is considerably lower than that of berries and grapes. Asami *et al*., 2003, reported that organically grown strawberries were found to have higher phenolics content than conventionally grown crops, though another study could not establish such a correlation (Hakkinen and Torronen, 2000). Processing and storage may have varying impacts on different phenolic compounds, as seen in berry processing where myricetin and kaempferol were found to be more prone to losses than quercetin (Hakkinen *et al*., 2000).

Table 1: Total phenolic compound (Antioxidant) of eight edible plants obtained from India and Iran

Samples	Total phenolic compound mg/100g
Alocacia indica Sch	87
Asparagus officinalis DC	317
Portulaca oleracia Linn	586
Momordica dioicia Roxb	396
Eulophia ochreata Lind	243
Solanum indicum Linn	702
Cordia myxa Roxb	402
Chlorophytum comosum Linn	136

Materials and Methods

Collection of samples: Eight different types of fruits and vegetables (*Alocacia indica Sch*., *Asparagus officinalis DC*., Chlorophytum comosum Linn., Cordia Myxa Roxb., Eulophia Ochreata Lindl., Momordica dioicia Roxb., Portulaca oleracia Linn. and Solanum indicum Linn.) were purchased from were collected from various localities of Maharashtra (India) and Iran. Five wild edible plants were collected from Iran viz *Asparagus officinalis*, *Chlorophytum comosum*, Codia myxa, Portulaca oleracia and Solanum indicum were collected from Iran in October 2006 and April 2007. Efforts made to collect these plants in flowering and fruiting conditions for the correct botanical identification. Healthy and disease free edible plant part/s selected Each variety of fruit and vegetables was collected to assess total phenolic contents.

Samples preparation: Fresh fruits and vegetables were cleaned with water and external moisture wiped out with a dry cloth. The edible portion of the individual fruits was separated, dried in a hot air oven at 50°C for 1 h. The dried samples were then powdered in blander for further study.

Some of the plants dried under shade so as to prevent the decomposition of chemical compounds present in them.

Determination of total phenolic compound

According to method singleton and rossi: Preparation of Plant Extracts (Method B). Grounded dry plant material (500 mg) was weighed into a test tube. A total of 10 mL of 80% aqueous methanol was added and the suspension was stirred slightly. Tubes were sonicated 5 min and centrifugated for 10 min (1500g) and supernatants were collected. Plant materials were re-extracted twice.

Determination of total phenolics: The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965). Samples (0.5 ml, two replicates) were introduced into test tubes; 2.5 mL of Folin-Ciocalteu's reagent and 2 mL of sodium carbonate (7.5%) were added. The tubes

were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as tanic acid equivalents in milligrams per gram dry material.

Results and Discussion

Results showed that total phenolic amounts of *Momordica dioicia Roxb* (396mg/100g) and of *Cordia myxa Roxb*(402 mg/100g) were comparable with total phenolic amount of Mint vegetable. (399.8 %), but the amounts were more than total phenolic amounts of other vegetables (Kaur and Kapoor, 2002). Total phenolic amount of *Solanum indicum Linn* (702 mg/100g) was more than total phenolic amounts of Black berry (417-555%) (Sellappan, 2002) and Cranberry (527.2%) (Sun, 2002).

Therefore, antioxidant capacity of *Solanum indicum Linn.* was high and antioxidant capacity of *Alocacia indica Sch.* was low.

Phenolic compounds could be a major determinant of antioxidant potentials of food plants and could therefore be a natural source of antioxidants and because Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables, Therefore, *Solanum indicum Linn.* has high preservation capacity and nutritional values, because total phenolic compounds prevent from damage of nutrients contain double bonds such fatty acids, flavor compounds even proteins and amino acids and other compounds.

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Baking and Storage Stability of Retinyl Acetate (Vitamin A) Fortified Cookies

Shahid Mahmood[1], Masood Sadiq Butt[1], Faqir Muhammad Anjum[1] and Haq Nawaz[2]
[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan
[2]Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan

Abstract: Cookies were prepared from commercially available straight grade flour using Retinyl acetate (RA) as fortificant @ 30, 40 and 50% of Recommended Daily Allowance (RDA). The product was packed in Bioriented poly propylene (BOPP) and analyzed on monthly basis for physico-chemical and sensory attributes including baking and storage stability of the fortificant up to three month. The results revealed the non-significant influence of fortification on physico-chemical composition and sensory characteristics and the cookies containing 50% of RDA; Retinyl acetate were found the best in overall acceptability. Baking loss of Retinyl acetate was 9.30% while 8.33% loss was observed during storage.

Key words: Fortification, Retinyl acetate, cookies, baking stability and storage stability

Introduction

Vitamin A deficiency (VAD) is a global nutritional problem particularly in the developing world that afflicts severely health of pregnant and lactating women, infants and children. In Pakistan about 60% of the males, 71% of the non-pregnant and lactating females and 79% of pregnant women consume less than 70% of the RDA of vitamin A (GOP, 1970).

Based on the above mentioned facts, it is highly probable that Pakistani females and children both in rural and urban areas are at significant risk of vitamin A deficiency. Enhancing vitamin A supplement can help to reduce child mortality by 25% (Sher, 2004).

There are three major strategies to combat Vitamin A deficiency, which are as food fortification, supplementation with vitamin A and food diversification (Bloem *et al*., 1998; Chakarvarty, 2000; Filteau and Tomikins, 1999). Food fortification can be an economical, flexible and socially acceptable way to improve the nutrient intake of groups at risk in order to ensure nutritionally adequacy of the diet (Hoffpauer and Wright, 1994). It is an option where people have access to milled or processed food (Mason *et al*., 2001). Fortification can also reinstate the natural vitamin A content of a foodstuff if it has been lost during processing. Vitamin A fortification requires special attention in regard that vitamin A is fat-soluble and proven vehicles for vitamin A fortification include sugar, oils and fats and cereals flours (Arroyave *et al*., 1979; Dary *et al*., 1996; Lotfi *et al*., 1996). Bauernfeind and De Ritter (1991) described cereals as best for fortification because in developing countries 95% of the population consumes cereals as dietary staple and according to Ranum (2002) as staples, milled cereals are relatively inexpensive, they are grown and consumed worldwide by all economic classes, versatile in preparation and use, generally processed in large centralized plant and milled cereals are better for fortification. In poor households, the overwhelming part of the diet might consist of staple grains and vegetables (Graebner *et al*., 2004).

There are three biologically active forms of vitamin A, i.e., Retinol, Retinal, and Retinoic acid. Retinol is the true form of vitamin A, found in the foods of animal origin, readily used by the human body. Retinyl esters are found in animal foods such as liver, eggs and whole milk (Trumbo *et al*., 2001).

The most important commercial forms of vitamin A are Retinyl acetate and Retinyl palmitate. These pure chemicals have mainly been added to foods as food improvers and colorants, but foods can also carry them to increase vitamin A intake of the populations consuming these foods (Lotfi *et al*., 1996).

Regarding the stability of vitamin A in cookies the previous studies of Rice *et al*. (1941) revealed that in baked products such as bread, biscuits and cake, which are baked under moderate conditions, it appears that 80 to 100 per cent of the vitamin A survives the baking process.

Vitamin A is sensitive to heat and light and higher storage temperature increases the oxidation of vitamin A and results in loss of Retinyl acetate's ability of binding with lipids and also lowers its absorption capacities (Butt *et al*., 2007).

The present study was designed to prepare vitamin A fortified cookies containing Retinyl acetate and to evaluate its stability during baking and storage.

Materials and Methods

Procurement of raw materials: Commercial straight grade flour was purchased from local market and the fortificant (Retinyl acetate) was purchased from Sigma Chemicals, Switzerland.

Table 1: Different treatments used to prepare fortified cookies

Treatments	Retinyl acetate % RDA
T_1	(Control)
T_2	30
T_3	40
T_4	50

Table 2: Effect of fortificant on physical parameters of fortified cookies

Treatments	T_1	T_2	T_3	T_4
Width (cm)	2.37	2.38	2.34	2.33
Thickness (cm)	0.52	0.52	0.53	0.53
Spread Factor (cm)	0.45	0.46	0.46	0.45

Preparation of fortified cookies: Fortified cookies were prepared having fortificant at different proportions by some modifications in the method described in AACC (2000) and stored at room temperature in Bioriented poly propylene (BOPP). The detail of treatments used for preparing fortified cookies is given in Table 1.

Baking stability of fortified cookies: Retinyl acetate (vitamin A) contents of cookies were determined through spectrophotometer according to the method of AOAC (1990) before and after baking. Vitamin A losses were computed in percentage to estimate baking losses.

Storage studies: The fortified cookies were evaluated for physical (Width, thickness and spread factor) and chemical (Moisture, crude proteins, crude fat, crude fibre and ash contents) composition at 0, 30, 60 and 90 days of storage according to the methods described in AACC (2000). The Retinyl acetate (vitamin A) contents of cookies were determined through spectrophotometer according to the method of AOAC (1990) at the same intervals. The cookies were analyzed by a panel of judges for color, tastes, flavor, texture and overall acceptability by following the procedures of Larmond (1977).

Results and Discussion

Physical analysis: Fortification showed non-significant effect on width, thickness and spread factor of cookies. The highest width (2.37 cm) was found in T_1 (Control) while the lowest (2.33 cm) in T_4 (50 % of RDA; RA). Regarding thickness, addition of Retinyl acetate slightly increased the value from 0.50 cm to 0.53 cm. However, no trend was observed in change of spread factor due to addition of the fortificant (Table 2).

Present studies reveal that fortification had no adverse effects on physical parameters of cookies. Storage of 90 days also had the significant influence on physical parameters. Only width of cookies slightly but non-significantly decreased from 2.38 cm (at 0 day) to 2.32 cm after 90 days (Table 3).

However, no obvious change was recorded in thickness and spread factor of fortified cookies during storage.

Table 3: Effect of storage on physical parameters of fortified cookies

Storage days	0	30	60	90
Width (cm)	2.38	2.35	2.37	2.32
Thickness (cm)	0.52	0.53	0.52	0.53
Spread Factor (cm)	0.46	0.45	0.46	0.45

Table 4: Effect of fortificant on chemical compositions (%) of fortified cookies

Treatments	T_1	T_2	T_3	T_4
Moisture	2.76	2.72	2.69	2.70
Ash	0.56	0.57	0.57	0.57
Protein	6.56	6.57	6.57	6.56
Fat	22.54	22.43	22.56	22.49
Fiber	0.26	0.25	0.26	0.26
NFE	67.27	67.41	67.34	67.36

Table 5: Effect of storage on chemical compositions (%) of fortified cookies

Storage days	0	30	60	90
Moisture	2.53^{c}	2.71^{b}	2.74^{b}	2.88^{a}
Ash	0.54	0.55	0.59	0.60
Protein	6.57	6.55	6.55	6.55
Fat	22.41	22.48	22.57	22.55
Fiber	0.25	0.26	0.26	0.26
NFE	67.70^{a}	67.43^{b}	67.29^{c}	67.04^{d}

Spread factor and size of the cookies depends on particle size and moisture content of flour (Gaines and Donelson, 1985).

Chemical composition: Regarding chemical composition, non - significant effect of fortification was found on all the parameter like moisture, ash, protein, fat, fiber and NFE (Table 4). Addition of Retinyl acetate slightly decreased the moisture level as control treatment had 2.76% moisture content while moisture was found 2.70 % in cookies containing 50% of RDA of Retinyl acetate.

However, no gradual change was observed in other chemical parameters on addition of the fortificant. The mean composition of cookies recorded in present study is in accordance with Butt *et al.* (2007) who found the similar composition in cookies.

The effect of storage was found significant on moisture and NFE content of fortified cookies while non-significant changes were observed in other parameters during 3 months. The moisture level increased from 2.53% (at 0 day) to 2.88% after 90 days and mean NFE content significantly decreased from 67.70% to 67.04% (Table 5).

Although some biochemical changes occurred during storage but do not affect the crude proximate composition. The increase in moisture may be due to moisture in air that affects the cookies even in packaging. Butt *et al.* (2007) also determined the significant increase in moisture content of vitamin A fortified cookies during storage.

Table 6: Effect of fortificant on sensory characteristics (scores) of fortified cookies

Treatments	T_1	T_2	T_3	T_4
Color	7.09[a]	6.90[b]	6.81[c]	6.48[d]
Flavor	7.00	6.90	6.95	6.67
Taste	6.86	6.90	6.95	7.14
Crispiness	7.00	7.05	7.09	7.24
Texture	7.05[bc]	7.00[c]	7.14[b]	7.43[a]
Overall acceptability	7.19	7.09	7.14	7.24

Table 7: Effect of storage on sensory characteristics (scores) of fortified cookies

Storage days	0	30	60	90
Color	7.25[a]	7.17[b]	6.67[c]	6.00[d]
Flavor	7.42[a]	7.25[b]	6.75[c]	6.00[d]
Taste	7.42[a]	7.24[b]	6.75[c]	6.33[d]
Crispiness	8.00[a]	7.25[b]	6.84[c]	6.17[d]
Texture	8.00[a]	7.42[b]	7.00[c]	6.25[d]
Overall acceptability	7.75[a]	7.33[b]	7.16[c]	6.16[d]

Sensory evaluation: Fortified cookies were evaluated for organoleptic characteristics after one month intervals. The fortification significantly influenced the color and texture of cookies. Fortified cookies were awarded slightly lower scores for color and flavor as compared to un-fortified cookies. However, taste, crispiness and texture were improved on addition of Retinyl acetate. The lowest scores (6.86, 7.00, 7.05) were awarded by T_1 (control treat) while the highest scores (7.14, 7.24, 7.43) were gained by T_4 (50% of RDA; RA) for taste, crispiness and texture respectively. Overall, the cookies containing 50% of RDA of Retinyl acetate (T_4) was liked the most (Table 6).The adverse effect of fortification on color and flavor of cookies was due to Millard's, reaction (Bender, 1996). However, these changes were under acceptable limits.

During storage, significant decrease was observed in scores awarded to fortified cookies for all the characteristics, however, after 3 months of storage the overall acceptability was under acceptable limits. The mean scores awarded for overall acceptability at 0 days were 7.75 that significantly decreased to 6.16 after 90 days (Table 7).

The decrease in sensory scores as function of storage might be due to oxidation of fats and Millard's reaction. Similar, findings were described by Bender, 1996; Elahi, 2006 and Wada, 1998 who also reported the same decreasing trend in color, flavor, taste, texture, crispiness and overall acceptability with storage. During storage absorption of moisture by cookies results in deterioration of color, flavor, crispiness and texture (Wada, 1998).The same reason may be linked with present findings as the moisture level of cookies significantly increased during 90 days of storage.

Effect of baking on Retinyl acetate: The results regarding vitamin A contents prior and after baking of fortified cookies as presented in Table. Results showed minor loss (9.3%) during baking (Table 8). The mean Retinyl acetate contents before baking were 10.99 which reduced to 9.96 µg after baking. The baking loss of vitamin A is similar to the findings of Emodi and Scialei (1980) who reported 7-10% and Butt *et al.* (2007) who investigated 8.69-11.11% loss during baking.

Table 8: Baking stability of Retinyl acetate (µg) in fortified cookies

Treatments	Before baking	After Baking
T_1	0.19	0.16
T_2	10.98	9.93
T_3	14.59	13.43
T_4	18.18	16.33
Means	10.99[a]	9.96[b]

Table 9: Storage stability of Retinyl acetate (µg) fortified cookies

Treatments	0	30	60	90	Means
T_1	0.16	0.15	0.13	0.11	0.14
T_2	9.93	9.62	9.33	9.03	9.48
T_3	13.43	13.08	12.74	12.35	12.88
T_4	16.33	15.92	15.48	15.02	15.69
Means	9.96[a]	9.69[b]	9.42[c]	9.13[d]	

Storage stability: Storage of cookies for 90 days at ambient temperature high significantly reduced the Retinyl acetate content (Table 9).

At start of study mean vitamin A contents were 9.96 µg which decreased to 9.13 µg after 90 days. The percent loss recorded during storage was 8.33%. As the storage period increased, the higher percent loss was recorded. Butt *et al.* (2007) recorded 10.8% loss in vitamin A content of cookies during one month of storage. Bauernfeind and Ritter (1991) also found slight loss in Vitamin A after six months storage.

At higher storage temperature, due to oxidation Retinyl acetate lose its ability of binding with lipids and also lowers absorption capacities (Butt *et al.*, 2007).

Conclusion: Fortification is considered the most effective strategy in combating vitamin A deficiency. The present study revealed that fortification of vitamin A did not influence the physico-chemical properties and sensory characteristics of cookies. During baking 9.3% and during storage 8.33% loss in RA was recorded. Hence, it was concluded that cookies fortified with RA @ 50% of RDA can be prepared and provided to the people to combat VAD.

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The Use of Different Sources of Protein on the Growth and Reproduction of Pigs

F.C. Iheukwumere[1], E.C. Ndubisi[2] and P.E. Etusiim[3]
[1]Department of Agriculture, Abia State University, PMB 7010, Umuahia, Abia State, Nigeria
[2]Department of Animal Science, Imo State University, PMB 2000, Owerri, Nigeria
[3]Department of Animal and Environmental Sciences, Abia State University, PMB 2000, Okigwe, Nigeria

Abstract: Eighteen clinically sound and parous sows and 6 boars of Large White x Landrace crosses were used to study the effects of different sources of protein on the growth and reproduction. The treatments T_1, T_2 and T_3 which contained T_1 Fish meal (FM) control, T_2 Chicken offal meal (COM) and T_3 Full-fat soybean (FFSB) and fed to the pigs in a completely randomized block design (CRD). The mean body weight gain, feed intake and feed conversion ratio did not differ significantly ($P > 0.05$) between treatment groups. However, higher numerical values were obtained for COM and FFSB treatment diets. The gestation length ranged from 114 – 116 days. The number of piglets born were similar ($P > 0.05$) between the treatment groups, but COM and FFSB treatment groups showed higher values. The piglet birth weight gain were similar ($P > 0.05$) between COM and FFSB treatment groups, however, they differed significantly ($P < 0.05$) from T_1 FM control. The piglets weight gain at 0- 2, 0 – 4 weeks of age were similar ($P > 0.05$) between treatment groups. However, piglets weight gain at 4 – 8 weeks of age were similar between COM and FFSB treatment groups but differed significantly ($P < 0.05$) from T_1 FM group. The number of piglets weaned and weaning weights were similar ($P > 0.05$) between treatment groups, however, higher values were observed in COM and FFSB treatment groups. It is concluded that COM and FFSB can completely replace fish meal in the rations of pregnant pigs without any deleterious effects on growth and reproduction.

Key words: Protein sources, growth, reproduction, pigs

Introduction

The high cost and adulteration of fish meal is becoming increasingly important to explore other alternative sources for providing dietary protein in monogastric animal feeding (Esonu *et al.,* 2001; Madubuike, 1992). Chicken offal meal (COM) is an abattoir waste whose potential as feed ingredient in monogastric rations is presently being investigated. Chicken offal meal is a product resulting from rendering of some poultry eviscerating parts mainly the gut contents. It is rich in protein, fat and minerals. Chicken offal meal has been established as a valuable ingredient in broiler diets, with growth promoting properties similar to those of fish meal. It has been used as a protein source in the diets of chickens (Salami and Oyewole, 1997) and pigs (Akpodiete *et al.,* 2001).

Full-fat soybean (FFSB) has proved to be a valuable alternative of plant protein source that can be used to replace groundnut cake, as well as reduce the conventional requirement of fish meal in monogastric animal feeds (Madubuike, 1992). If properly processed, full-fat soybean can make a particularly useful contribution to the diets of pigs by virtue of a high protein content with good acid balance, a large proportion of oil, which over half is the poly-unsaturated essential fatty acid, linoleic acid and an ability to mix rapidly and uniformly with other dietary ingredients (Aburto *et al.,* 1998). According to Bowers *et al.* (2000), it was possible to achieve levels of performance in pigs which were higher with the use of FFSB than those with more traditional diet based on skimmed milk and fish meal. This study was designed to evaluate the effect of different protein sources on the growth and reproduction of sows.

Materials and Methods

Management of experimental animals: Eighteen clinically sound and parous sows and 6 boars, large White x Landrace were used for this study. The pigs were housed in concrete floored pens equipped with feeding and watering troughs. Feed and water were provided *ad libitum*. The experiment lasted for 36 weeks. Pigs weight and feed consumption were recorded weekly throughout the 12-week feeding trial.

Preparation of the protein meal: The full-fat soybean was processed by lowering a raw soybean packed in jute bags in a half-drum of boiling water and allowed to boil for 60 minutes. Timing of the boiling commenced when the water reached 100°C after introducing the bags. The boiled seeds were drained of water and sundried to less than 10% moisture level before being ground for use.

The chicken offal meal was got from the broiler chicken intestines (gut content inclusive). They were cleaned of residues and washed with water to remove dirt. These

Table 1: Proximate chemical composite of the protein sources (g/kg)

Nutrients	FM	COM	FFSH
Moisture	80.00	118.00	82.10
Crude protein	656.00	600.00	430.00
Crude fibre	10.00	61.10	64.80
Ether extract	77.00	84.60	185.00
Nitrogen free extract	161.00	110.30	274.80
Ash	96.00	144.00	45.40
Minerals			
Calcium	51.00	8.10	5.91
Magnesium	3.40	2.30	0.61
Sodium	1.80	20.06	26.67
Potassium	3.50	12.02	10.48
Phosphorus	30.00	5.10	0.86
Iron	0.30	0.08	0.33

FM = Fish meal, COM = Chicken offal meal, FFSB = Full-fat soybean

Table 2: Composition of experimental diets (g/kg)

	FM	COM	FFSH
Maize	450.0	445.4	425.0
Maize offal	250.0	250.0	250.0
Fish offal	25.0	-	-
Chicken offal meal	-	27.1	-
Full-fat soybean	250.0	250.0	300.0
Bone meal	12.5	12.5	12.5
Oyster shell	7.5	7.5	7.5
Premix*	2.5	2.5	2.5
Salt	2.5	2.5	2.5
Determined (DM basis)			
Crude protein	171.0	177.0	180.0
Crude fibre	46.0	53.0	40.0
Ether extract	40.0	51.0	40.0

*Supplied the following per kg of ration: Vit A, 11,785 IU; Vit. D, 1944.3 IU; Riboflavin, 5.4 mg; Panthotenic acid, 9.82 mg; Nicotinic acid, 24.55 mg; Choline chloride, 4.0 x 10^5 mg; Vit E, 4.91 IU; Vit. B_{12} 0.01 mg; Methionine, 245.53 mg; Cobalt, 1.23 mg; Iodine 0.98 mg; Cu, 9.82 mg; Mn., 55.0 mg; Zn, 49.11 mg and Fe, 19.64 mg.

were then transferred into a half-drum, where the "wet rendering" method of processing was applied by cooking the intestines at a temperature of about 150°C for 2 hours. The broth was allowed to cool, partially defatted by decanting the oil at the top layer and further pressed gently to expel oil and water. The sample was sun-dried to less than 10% moisture level and ground for use. The chemical composition of the protein sources were determined by the methods of AOAC (1995).

Experimental design and procedure: The eighteen sows were divided into 3 treatment groups identified as T_1, T_2 and T_3. Each treatment group consisted of 6 sows and was further replicated 3 times with two sows per replicate. Three diets were formulated, the control T_1 contained fish meal, T_2 contained COM and T_3 FFSB with total replacement of the fish meal with COM and FFSB respectively on protein basis. The diets were isonitrogenous and caloric with 18% CP and 2900 kcal ME/kg and randomly allotted to the treatment groups. All the sows were served by the respective boars attached to the treatment group. The dates of mating and farrowing of the pregnant pigs were recorded. The following parameters were evaluated. 1. Gestation length. 2. Number of piglets born. 3. Number of piglets born alive 4. Weight of piglets. 5. Number of piglets weaned. 6. Piglets weaning weights.

Data analysis: All the data collected from this study were subjected to analysis of variance Steel and Torrie (1980) and treatment means where significant were separated using Duncan's New Multiple Range Test as described by Obi (1990).

Results and Discussion

The results of the Chemical Composition of the protein sources are shown in Table 1, the proximate composition of the experimental diets are shown in Table 2, growth performance is shown in Table 3 while the reproductive performance of the sows are shown in Table 4. The growth performance of pigs fed on the different sources of protein did not show any significant differences ($P > 0.05$) between the treatment groups. The weight gain FM 0.51 (kg/day); Com 0.58 (kg/day) and FFSB 0.59 (kg/day) were not significantly different ($P > 0.05$) between the treatment groups. Feed intake, FM 1.62 (kg/day) COMM 1.55 (kg/day) and FFSB 1.56 (kg/day) were not significantly different ($P > 0.05$) between treatment groups. The feed conversion ratio (feed/gain) showed that pigs fed on FM diets had 2.75; COM 2.63 and FFSB 2.85. However, COM showed a better feed conversion ratio. Protein sources had no significant effect ($P > 0.05$) on the growth performance of pigs, although FM slightly improved the performance. These observed results are consistent with the findings of Bamgbose and Kudi (1996), Salami and Oyewole (1997) who reported that chicks fed COM had comparable performance to those fed the animal protein-free control diet. Also Akpodiete and Ologhobo (2000) found COM to be effective as FM when used to supplement a simplified corn-soya ration. The results confirmed the possibility of replacing fish meal with FFSB in pig diets Udedibie *et al.* (1987).

The results of the effect of different sources of protein on the reproductive performance of pigs are shown in Table 4.

The litter size of the pigs fed on diet containing FFSB was 12.00, COM 10.00 and FM 8.00. These values were similar ($P > 0.01$) between the treatment groups. The highest litter size of 12.00 observed in this study was higher than the range 7.11 ± 0.78 – 7.46 ± 0.45 reported by Orheurata (2000) in pigs. The piglets birth weight were significantly different ($P < 0.05$) between treatment groups. Pigs fed on FFSB diet 1.43 kg, COM 1.40 were similar ($P > 0.05$) but they differed significantly ($P < 0.05$)

Table 3: Effect of different protein sources on the growth performance of pigs

Parameters	Treatment (Protein sources)			
	T_1 FM	T_2 COM	T_3 FFSB	SEM
Initial live weight (kg/pig)	24.30	24.20	24.10	0.26
Final live weight (kg/pig)	61.40	58.20	59.00	0.30
Weight gain (kg/day)	0.51	0.58	0.54	0.05
Feed intake (kg/day)	1.62	1.55	1.56	0.16
Feed/gain	2.75	2.63	2.85	0.18

Means on the same row followed by the same superscripts are not significantly different (P > 0.05). FM = Fish meal, COM = Chicken offal meal, FFSB = Full-fat soybean

Table 4: Effect of different protein sources on the reproductive performance of pigs

Parameters	Treatment (Protein sources)			
	T_1 FM	T_2 COM	T_3 FFSB	SEM
Gestation length (days)	114.0	116.0	116.0	0.99
Litter size	8.00	10.00	12.00	2.46
Liter birth weight (kg)	1.32^b	1.40^a	1.43^a	0.02
Piglet with gain (kg)				
0 – 2 weeks	2.66	2.70	2.73	0.08
2 – 4 weeks	2.74	3.15	3.05	0.47
4 – 8 weeks	5.20^b	5.79^a	5.78^a	0.13
No. of piglets weaned	7	9	11	3.47
Weaning weight of pig (kg)	5.80	6.98	6.59	0.34
Mortality	1	1	1	

from pigs fed on FM diet 1.32 kg. These values in litter weight were comparably lower than 5.60 ± 0.17 reported by Orheurata (2000) in pigs. The differences in litter weight observed in their study many be due to breed differences (Belstra *et al*., 1997).

It is observed that with increase in litter size, birth weight, decreased (Orheurata, 2000). An interesting observation that can be made from this study was that there was an inverse relationship between litter size and litter weight. This observation is in agreement with the findings of Barker and Chung (1992), Mahan and Grifo (1995).

The piglets weight gain at 0 – 2 weeks of age were for FM diet 2.66 kg, COM diet 2.70 kg while FFSB diet was 2.73 kg. Higher numerical values were obtained in pigs fed COM and FFSB diets, however, they were not significantly different (P > 0.05) from FM treatment diets. Piglets' weight gain at 2 – 4 weeks of age followed the same pattern as in the 0 – 2 weeks of age. However, at 4 – 8 weeks of age, the piglets' weight gain for FM diets was 5.20 kg, COM diets 5.79 kg and FFSB diets 5.78 kg; these values for COM and FFSB diets differed significantly (P < 0.05) from FM diet. The observations in piglets weight as affected by age is in agreement with the reports of Belstra *et al*. (1997) who indicated that piglets weight gain increased with the age of the piglets.

The number of piglets weaned from FM diet 7.0, COM diets 9.0 and FFSB diet 11.0. These values were similar (P > 0.05) between the treatment groups. The weaning weight followed the same trend as in the number of piglets weaned. The higher weaning weight observed in COM treatment diet in this study was comparably higher than 4.87 – 5.58 range reported by Orheurata (2000) in pigs. The observed differences in this study may be attributed to breed differences Speer (1990), nutrition and physiological status of the animal. Esonu *et al*. (2001).

The results show higher gestation length for COM and FFSB diets of 116 days for each group of pigs. A lower gestation length of 114 days was observed in pigs fed the FM diet. The observed gestation lengths in this study ranged from 114 – 116 days. This value falls within the range 113 – 118 reported by Orheurata (2000) n pigs. Susan Higginson (1998) indicated that pigs with higher gestation length manifested improved reproductive performance. This observation is in agreement with the results obtained in this study.

The mortality of the piglets showed no significant differences (P > 0.05) between the treatment groups. The similarity in piglet mortality observed in this study may be attributed to fewer deaths from crushing and that the piglets were better at escaping from under the sow. This finding is in agreement with the reports of Susan Higginson (1998).

Conclusion: The results from this study suggest that fish meal can be completely replaced by chicken offal meal or full-fat soybean in the rations of pregnant pigs for improved growth and reproductive performance.

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Effect of Mixed Feeding Regime on Litter Performance Traits of Rabbit Does

F.C. Iheukwumere
Department of Animal Science and Fisheries, Abia State University, PMB 7010, Umuahia, Nigeria

Abstract: Twenty-four clinically sexually mature New Zealand white rabbits consisting of (4 buck and 20 does) were used to study the effect of concentrate and Talinum triangulare combinations by breeding does during pregnancy on litter performance traits. The treatments comprised the following concentrate and Talinum triangulare combinations (%) respectively: (1) 20:80, (2) 40:60, (3) 60:40, (4) 80.20.. A total of 150 g/day was offered to the does during pregnancy. Average litter sizes at birth and weaning and litter weaning weight were similar ($P > 0.05$) between 40:60 and 60:40 concentrate and forage combinations, but, they differed significantly ($P < 0.05$) from 20:80 and 80:20 concentrate and forage combinations. However, 20:80 diets differed significantly ($P < 0.05$) from 80:20 diet combinations. Average litter weight gains (0 – 35 days) for the various concentrate and forage levels were 2054.40 ± 14.25 g (20:80), 2270.74 ± 18.85 g (40:60), 2314.40 ± 24.64 g (60:40) and 1485.24 ± 19.30 g (80:20). Mortalities were not significantly different ($P > 0.05$) between diets. From the economic stand point therefore, diet with 60:40 concentrate and forage combinations could be considered optimum, based on result on litter sizes, litter weight at weaning and mortality.

Key words: Mixed feeding, does, litter traits

Introduction

Nigeria like many other developing countries of the world has a protein deficiency gap, especially that of high quality animal protein. The inadequate supply of protein from the traditional livestock – cattle, sheep, goat and chicken has led to the intensification of efforts to improve on the productivity of these animals. (Rabbit has been thought of as being suitable in this regard (Iheukwumere and Okoli, 2002). The most advantageous attribute of rabbits is their high reproductive potential (Lebas *et al*., 1997). This is as a result of their short gestation length, early sexual maturity, high prolificacy and ability to rebreed shortly after parturition all leading to a short generation interval (Effiong and Wogar, 2007).

Despite the above advantages, studies on the productive system showed that among other factors, feeding is one of the major limiting factors in achieving maximum performance in rabbits (Iheukwumere *et al*., 2005). Asuquo (1993) observed that poor nutrition will delay sexual maturity in rabbits resulting in low financial returns for the farmer. Lebas (1993) and Effiong and Wogar (2007) also observed that increased feed and nutrient levels have been advocated for breeding rabbits as a means of increasing litter size, adequate maintenance of pregnancy and subsequent milk let down by the does. Aduku and Olukosi (1990) observed that even though forages can support up to 50% of rabbit nutrient requirements, these animals cannot be fed forages for optimum performance. Aliyu (1990) also observed that breeding rabbits on sole forage alone cannot guarantee maximum productivity considering the limited forage utilization capacity of this herbivore.

Table 1: Proximate Composition of Concentrate and Talinum triangulare fed to pregnant does

Nutrients	Concentral meal (%)	Talinum triangulare (%)
Dry matter	95.56	1.10
Ash	6.54	2.50
Ether extract	13.15	0.40
Crude fibre	9.18	1.70
Crude protein	22.85	2.50
Nitrogen free extract	47.63	2.00

Ngodigha and Mepha (1992) noted that feeding concentrate along side forages will improve reproductive efficiency of breeder rabbits.

This study was therefore designed to evaluate the feeding value of concentrate combined with forage Talinum triangulare on litter performance traits of breeding rabbits under mixed feeding regime.

Materials and Methods

Twenty-four sexually mature and clinically sound New Zealand white rabbits consisting of (4 bucks and 20 does) were used for this study. The rabbits were raised at the Rabbitary Unit of the Livestock and Research Farm, Abia State University, Umuahia, Nigeria. The rabbit does were divided into four treatment groups consisting of 5 does per treatment group. Each treatment group was replicated 5 times with one doe per replicate raised individually on separate cages. The treatments comprised the following:

Concentrate and Talinum triangulare combinations (%) respectively (1) 20:80, (2) 40:60, (3) 60.40 and (4) 80:20 fed to the rabbits in a completely randomized design.

Table 2: Reproductive performance of rabbit does fed concentrate and Talinum triangulare combination diets

Parameters	Treatment (Concentrate: Forage)			
	20:80	40:60	60:40	80:20
Kindling status (%)	51[ab]	65[ab]	77[a]	38[5]
Average litter size at birth	5.85[b] ± 0.24	6.18[a] ± 0.20	6.25[a] ± 0.30	5.00[c] ± 0.32
Average litter birth weight (g)	261.85[b]±10.65	273.42[a]±14.01	311.45[a]±13.33	266.23[c]±11.04
Average litter size at weaning	4.60[b] ± 1.30	5.13[a] ± 0.75	5.22[a] ± 1.21	3.65[c] ± 1.42
Percentage mortality (Pre-weaning)	15.31	14.65	13.25	18.24
Average quantity of daily conc. + forage intake (g)	95.06[c]	136.12[a]	143.54[a]	113.61

[b]a' b' c: Means with different superscripts along rows are significantly different . ($P < 0.05$)

The concentrate used was pelleted poultry grower's mash meal (20% CP and 2700 Kcal/kg) routinely fed to the rabbits. Talinum triangulare was harvested and dried at room temperature and chopped before feeding. The concentrate and forage were fed in the morning at 08.00 hour in separate feeders. A total of feed supply of 150 g/day was offered the does during pregnancy. Water and mineral lick were supplied *ad libitum*.

Random mating of the does to the bucks was done between 8.00 – 11.00 am and two weeks post partum. Pregnancy diagnosis was done by palpation and weight method as described by Iyeghe-Erakpotobor *et al.* (2007). The does were supplied earthen nest pots on day 25 of pregnancy. At kindling the kits were counted and weighed. Kittens were weaned at 35 days of age. The parameters monitored were litter sizes taken 24 hours after kindling, weights at birth and weaning. The proximate composition of the concentrate and Talinum triangulare was analyzed according to AOAC (1995). This study lasted for 4 months.

Data analysis: All the data collected from this study were subjected to analysis of variance (Steel and Torrie, 1980) and means separated using Duncan's New Multiple Range Test as described by Obi (1990).

Results and Discussion

Table 1 shows the proximate composition of concentrate and Talinum triangulare diet combinations, while Table 2 shows the results of the performance litter traits of breeder rabbit does on mixed feeding regime.

The results on average litter sizes at birth showed that rabbit does fed on 40:60 concentrate and forage diets 6.18 ± 0.20 and 60:40 concentrate and forage diets 6.25 ± 0.30 were not significantly different ($P > 0.05$) but they differed significantly ($P < 0.05$) from 20:80, 5.85 ± 0.24 and 80.20 5.00 ± 0.32 concentrate and forage combination diets.

In this study, it was observed that litter sizes at birth increased up to 60.40 concentrate and Talinum triangulare combination diet, but then decreased at 80:20 concentrate and forage combination diets. The higher performance of does on this treatment as expected was not achieved in this study. This observation agrees with the findings of Iyeghe - Erakpotobor *et al.* (2007) in rabbits does fed concentrate and *stylosanthes hamata* combinations during pregnancy. However, the significant differences observed in the litter sizes at birth in this study disagrees with earlier reports of Iyeghe-Erakpotobor and Muhammad (2004) who did not observe any significant differences in litter size at birth for rabbit does fed varying levels of concentrate and *lablab* combinations. Differences observed for litter size and weight at birth were due to differences in concentrate and forage levels (Asuquo, 1996). Increased concentrate and forage intake induced the shedding of more ova during ovulation, thereby increasing litter size at birth (Olentine and Esminger, 1980; Aduku and Olukosi, 1990). This observation has a strong basis on the fact that flushing of litter-bearing animals before breeding would enhance the number of young per litter (Asuquo, 1993; Odoh *et al.*, 2007). Also the linear relationship noted between concentrate and forage combination diets levels and litter weight could be argued of increased nutrient supply that enhanced better embryonic development pre-partum (Asuquo, 1996; Effiong and Wogar, 2007). Diets 40:60 and 60:40 concentrate and forage combinations, similarly promoted the highest average litter size and weight at weaning with no statistical differences ($P > 0.05$) between their values. Generally, for all parameters evaluated, performance declined at 80:20 diet combinations except in the concentrate and forage intake where the feed intake was higher in the 80:20 combinations 113.01 g and differed significantly ($P < 0.05$) from 20:80 combination diets 95.06 g. This observation is in agreement with the reports of Iyeghe-Erakpotobor *et al* (2007) in pregnant rabbits fed concentrate and stylosanthes combinations.

Average pre-weaning litter weights gains, based on litter weight at birth and weaning were (diets 20:80) 261.85 ± 10.65 g (diets, 40:60) 273.42 ± 14.01g, (diets60:40) 311.45 ± 13.33 g and (diets, 80:20) 206.23 ± 11.04 g. Percent mortality rate was lowest on 40:60 diet combinations.

Results form this study support the general view that a mixed feeding regime of concentrate-forage with the right proportion of concentrate and forage will maximize

litter traits in breeding rabbits. Diets 40:60 and 60:40 combinations of concentrate and forage combinations gave similar results in all traits.

Conclusion: It could be concluded that even though 40:60 and 60:40 concentrate and forage combination diets were similar in all traits determined, however, 60:40 combination diets showed higher values on litter sizes, litter weight at weaning and mortality. From the economic point of view 60:40 concentrate-forage combination diet can be considered optimal for breeding rabbits under the humid tropical environment.

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Effects of Cottonseed Cake Based Diets on Performance and Egg Quality Characteristics of Layers

G.O. Adeyemo and O.G. Longe
Department of Animal Science, University of Ibadan, Ibadan, Oyo State, Nigeria

Abstract: The study evaluated the performance of layers fed on diets in which Cottonseed cake (CSC) replaced Soybean cake (SBC) in five experimental rations such that 0% (control), 15%, 30%, 45% and 60% of CSC replaced SBC. The design of the experiment was completely randomized design (CRD). Chemical analysis was carried out to determine the crude protein (CP) and gossypol contents of CSC. In this experiment, seventy-five 23 week - old layers were fed with experimental layer diets for 12 weeks. Parameters evaluated include hen-day production (HDP), Feed conversion ratio (FCR), egg weight (EW) and haugh units (HU). All data were analyzed using descriptive statistics and analysis of variance. The determined CP of CSC was 35.11% and its gossypol content was 570g/ton. FCR ranged from 1.6 to 4.9, HDP from 47% to 68%, EW from 47.5 to 62.8 g, and HU from 3.1 to 6.7. Layers on 60% CSC replacement for SBC had higher values for the parameters measured which were not significantly different from the control. CSC can replace up to 60% SBC without adverse effects on performance and egg quality characteristics of laying birds.

Key words: Cottonseed cake, egg quality, layers

Introduction

In poultry production, diets are formulated to supply the nutrients needed by chickens to grow and lay eggs. These diets are composed of ingredients whose components can be digested and absorbed by chickens (Card and Nesheim, 1975).

Oilseed cakes have been reported to represent potential sources of protein and energy in poultry feeding. However, the cost of finished feeds containing the well known cakes like soyabean cake and groundnut cake has become prohibitive because of competition for the oilseeds by man. Therefore, it became necessary to find suitable plant protein alternatives less competed for by man. Cottonseed cake comes in as a useful alternative because of its relatively high crude protein content, cheapness and ready availability.

Cottonseed cake has not been extensively used in diets of egg-type chickens because of its potential deleterious effect on egg quality. Reid *et al*. (1987) reported that the presence of gossypol in CSC fed to layers resulted in egg yolk discolouration during storage.

Feeding trials on the use of CSC in chicken diets shows that it is necessary to reduce the content of gossypol to fairly low levels in order to avoid the unfavourable physiological effect of this anti-nutrient (National Animal Production Research Institute Report, 1984)

Secondly the protein quality of CSC is usually lowered because of the heat processing methods which reduce lysine availability, especially in the presence of free gossypol which causes the formation of a protein-complex limiting the digestion of protein.

This study was designed to provide further information on the utilization of CSC in egg-type chickens when incorporated as a substitute for soybean cake in layer diets. Effect on performance and egg quality were evaluated.

Materials and Methods

Experimental diets and birds: A total of 75 exotic ISA-Brown adult egg-type chickens of 23 weeks of age were randomly allotted to five dietary treatments each group having 15 layers. The experiment lasted 12 weeks. Cottonseed cake (CSC) was incorporated at 0.0, 4.53, 9.10, 13.60 and 18.13% into a basal diet which served as control which represented 0, 15, 30, 45 and 60% replacement of SBC with CSC respectively. The five diets were formulated to provide approximately 2.60 kcal ME.

Performance characteristics: Weekly records of feed intake were taken from which, the average weekly feed intake was determined. The number of eggs laid per day by the birds was taken from which, the average hen-day production was determine.

The weight of the eggs for each group was taken using a sensitive digital top loading balance. Feed conversion ratio was calculated using the following formula:

Feed Conversion Ratio = Average Feed Intake / Average Egg weight

Physical characteristics of eggs: Egg Shape Index was calculated by finding the ratio of width to the length of eggs. The egg width and length were measured using a vernier caliper. Yolk index was calculated by dividing the yolk height by the yolk width; both were measured with the vernier caliper.

Shell thickness was calculated by getting the average of

the measurements of the narrow, middle and broad end of the shell which was taken using a micro-meter screw gauge. The haugh unit was calculated by determining the logarithm of albumen height. The height of the albumen was measured at its widest expanse and mid way between the yolk edge and the external edge of the thick albumen using a tripod micrometer. The formula used in calculating the haugh unit was

$$100 \log (H + 7.57 - 1.7 W^{0.37})$$

Where H = albumen height and W is the weight of Egg.

Chemical and statistical analyses: The cottonseed cake and experimental diets were analyzed according to A.O.A.C (1995) for their proximate composition. The experiment was a completely randomized design. All the experimental data were subjected to the analysis of variance using SAS (1999) and where statistical significance was observed, the mean values were separated using the Duncan Multiple Range Test (Duncan, 1980).

Results

The determined proximate composition of the diets fed in the trial is presented in Table 2.

Nutrient composition: The determined crude protein ranged between 16.73% (diet 5) and 17.61% (diet 3). Crude fibre increased from 6.00 (diet 1) to 9.00% (diet 5), ether extract also increased for 0.50% (diet 1) to 5.00% (diet 5). Ash values also increased from 8.00% (diets1 and 3) to 10% (diet 4).

Performance characteristics of layers fed CSC based diets: There were no significant differences (P>0.05) in the feed intake of layers fed CSC based diets. The feed intake increased from 138g/day/bird fed diet 1 (control) to 148g/day/bird fed diet 5 (Table 3). The Feed Conversion Ratio did not follow any trend. Diet 2 had the best FCR of 2.46, even though this was not significantly different from diet 1 (control) with 2.56 or diet 5 with 2.63. Table 3 shows the values obtained for the live weight changes of layers fed CSC based diets. Birds in diet 4 had the highest increase in weight gain of 170 g gain while the least value came from diet 1 (control) which gave no increase in weight gain (0.0g).

The values of hen-day production are shown in table 3. Birds fed diet 2 gave the highest value of 80.5 % which was not significantly different (p>0.05) from diet 1 (control) which gave the lowest value of 72.8%.

Table 4 shows the results on egg weight of layers fed CSC based diets. The test diets did not differ significantly (p>0.05) in their effects on the egg weight of the birds. The highest value came from diet 3 with 56.1g while the lowest value was from diet 4 (54.8g) indicating that no particular trend of numerical differences was obtained.

Egg quality characteristics of layers fed CSC based diets: Egg quality characteristics of layers fed CSC based diet from 23 – 35 weeks of age are presented in Table 4.

Birds that were fed diet 2 gave the lowest value of shell thickness (0.21mm), while diets 3 to 5 gave the same value of 0.24mm. The differences between the shell thickness of eggs from the different dietary treatments were not significant (p>0.05).

The values of yolk index of eggs laid by birds fed with CSC based diets. The highest yolk index (0.54) was obtained in birds fed diet 3 while those fed diets 1 and 2 had the lowest value of 0.51. Other group value was 0.53 for both diets 4 and 5. No significant differences (p>0.05) were obtained between treatment means.

Table 4 shows that there were no significant differences (p>0.05) in the mean values of egg shape index across the treatment groups. The lowest egg shape index (0.75) was obtained in eggs produced from birds fed diet 2. Those fed diets 4 and 5 gave the highest egg shape index of 0.78. While value for others treatments were within these range.

The values of albumen height from eggs of birds fed the different dietary treatment are shown in Table 4. Birds fed 18% CSC gave the lowest value of 1.30, while birds fed the lowest level of CSC gave the highest value of 1.42. Other groups gave values ranging between 1.36 and 1.41. Values were not statistically significant (P>0.05).

Table 4 shows the treatment mean values of haugh unit for the diets. No significant treatment effects were obtained. The highest value 4.60 was obtained for diet 3 while the lowest value was from diet 4 (4.17). Other values ranged between 4.26 and 4.54.

Discussion

Chemical composition: All the experimental diets, met the nutrient requirements for layers as recommended by NRC (1994). Ryan *et al.* (1986) also reported values of 46.8% crude protein 6.0% moisture, 14.0% crude fibre, 0.9% ether extract and 6.5% ash. While Reid *et al.* (1987) reported chemical composition of 44.5% crude protein, 0.15% ether extract, 8.83% moisture.

Performance characteristics: Although the amount of feed consumed did not indicate any significant response to the treatment effects on the layers, a relatively higher consumption was recorded as the CSC level in the diet increased. The inclusion of CSC in the experimental diets correspondingly increased the fibre content as the level of CSC increases. Increased dietary fibre level is often associated with faster rate of passage which inhibits optimal benefit from feed intake through the gut filling effect with a consequential reduction in the feed consumption (Thorne *et al.*, 1991). The observed increase in feed intake as CSC level increases may be

Table 1: Composition of Experimental CSC-based Diets fed to Layers

	---------- % Replacement of SBC protein with CSC ----------				
	0%	15%	30%	45%	60%
Ingredients	1	2	3	4	5
Maize	46.00	47.00	48.00	49.00	52.00
Soyabean cake	18.00	15.30	12.60	9.90	7.20
Cottonseed cake	-	4.53	9.10	13.60	18.13
Corn offal's	13.70	10.00	8.50	8.74	5.50
Wheat offal's	9.71	10.58	9.21	6.17	4.58
Fish meal	1.50	1.50	1.50	1.50	1.50
Bone meal	3.00	3.00	3.00	3.00	3.00
Oyster shell	7.50	7.50	7.50	7.50	7.50
Layers premix	0.30	0.30	0.30	0.30	0.30
Methionine	0.02	0.02	0.02	0.02	0.02
Salt	0.27	0.27	0.27	0.27	0.27
Total	100	100	100	100	100
Calculated					
Crude protein (%)	16.41	16.39	16.28	16.21	16.11
M.E.(kcal/g)	2.60	2.55	2.56	2.55	2.59
Crude fibre	4.30	4.45	4.65	4.82	5.09

•Premix supplied per kg of diet: Vit A, 20,000 IU; Vit D, 2,000 IU; Vit E, 5 IU; Vit K, 5mg; Vit B_{12} 7.5mg; Riboflavin, 4.2mg; Pantothenic acid, 25mg; Nicotinic acid, 45 mg; Folic acid, 0.5mg; Cu, 35mg; Mn, 250mg; Zn, 125mg; Fe, 100mg; Iodine, 800mg; Cobalt, 1.25mg; Choline, 750mg

Table 2: Mean proximate composition of experimental diets and test ingredient

Diets	% DM	% CP	% EE	% CF	% ASH	% NFE
1	83.27	17.11	0.50	6.00	8.50	67.89
2	82.80	17.47	2.00	7.00	8.00	65.53
3	84.80	17.61	2.00	8.00	8.00	64.39
4	84.47	17.16	4.50	8.50	10.00	59.84
5	84.10	16.73	5.00	9.00	9.50	59.77
CSC	90.15	35.11	3.00	17.00	3.50	41.39

due to the natural instinct of the layers to eat to meet their energy needs. The increasing rate of inclusion of CSC inadvertently lowered available dietary energy. (Fetuga, 1984, Summers and Leeson, 1986; Gous *et al.*, 1990 and Fahey *et al.*, 1992). Therefore, the propensity of birds to consume more at higher dietary fibre gave a clear indication of the natural reactions to events of lower energy. The high fibre content of CSC prevented 100% replacement of SBC with CSC to ensure diets do not have more than 5% fibre level recommended for layers. FCR values increased in birds fed increasing amount of CSC in the experimental diets (Table 1). This could be explained by the higher feed intake in relation to the egg weights of the birds. Card and Nesheim (1975) reported that poor feed conversion in egg laying ventures can be an indicator of the poor quality of feed given to the birds. Mamputu and Buhr (1991) also observed high FCR in layers fed varying amount of oil seed cakes. Dietary treatment did not influence the FCR significantly.

The body weight gain did not show any significant difference. Of interest however, is the trend of slight increase observed among the groups fed CSC inclusions in their diets as compared with the control which had no CSC. The body weight change at the termination of the experiment was nil. Stated more clearly, the CSC based group birds had better gain than those without CSC, which though were not significant but as reported by Zelenka *et al.* (1984); Leeson *et al.* (1991); Lewis *et al.* (1994), Yannakopulos *et al.* (1995), a critical body fat content before the onset of lay was important because it profoundly influences the size of egg, no of eggs and the ability of the birds to withstand the stress associated with the process of egg production. However, the slight increase in weight by birds fed CSC was not reflected in their egg output as it did not differ significantly from birds without CSC.

Generally, the body gain of layers on the test diets obviously manifested the adequacy of the respective diets. The gain in weight almost followed the pattern exhibited by the feed consumed. Contrary to reported cases (Ochetim, 1987; Panigrahi *et al.*, 1987 and Dhara *et al.*, 1994) a slight but non-significant increase in body gain was recorded with CSC inclusion in diets. This observation was perhaps influenced by the residual lipid content in CSC; this alongside with the adequacy in the lysine and methionine levels of the ration may have been responsible for a better utilization of the CSC based diets. This agrees with findings of Longe and Adekoya (1988), Zatari and Sell (1990) and Panigrahi and Powell (1991) who reported higher feed intake and gain as a result of lipid/fat addition in poultry rations.

The results on percent hen-day-production shows that HDP% was not significantly influenced by the CSC based diets. The HDP% values obtained in this study

Table 3: Performance of layers fed CSC based diets at 23-35 weeks of age

	Treatments					
Parameters	1	2	3	4	5	SEM
Initial body wt(kg)	1.51	1.52	1.47	1.43	1.50	0.04
Final body wt(kg)	1.51	1.58	1.58	1.60	1.54	0.04
Feed intake (g/bird/day)	138.00	143.00	140.00	142.00	148.00	18.00
Bodyweight gain (g)	0.00	60.00	110.00	170.00	40.00	20.00
Hen-Day Production (%)	72.83	80.46	77.73	73.05	78.10	7.59
Feed conversion ratio (FCR)	2.56	2.46	2.51	2.57	2.63	0.26

Table 4: Egg quality characteristics of layers fed CSC based diets from 23 to 35 weeks of age

	Treatments					
Parameters	1	2	3	4	5	SEM
Shell thickness (mm)	0.22	0.21	0.24	0.24	0.24	0.06
Yolk Index	0.51	0.51	0.54	0.53	0.53	0.08
Egg shape index	0.76	0.75	0.77	0.78	0.78	0.03
Albumen height(mm)	1.36	1.42	1.39	1.41	1.30	0.11
Egg weight (g)	54.84	55.53	56.12	54.76	55.72	1.72
Haugh unit	4.37	4.54	4.60	4.17	4.26	0.80

72.8, 80.5 77.3, 73.1 and 78.1 were similar to values recorded by Obida and Stanford (1988) who reported 79.1, 77.4, 78.7 and 78.6 HDP% when caged layers were fed millet and sorghum grain as replacement for maize. Longe and Adekoya (1988) also recorded similar values when they fed palm kernel meal in a maize – groundnut meal based diets.

Furthermore, the values recorded in this study were higher than values recorded by Udedibie *et al*. (1988) 60.9, 64.4, 66.8 and 65.3 for hen-day production (%) when poultry offal meal was fed as protein supplement to 54 weeks old Hyline layers diets at 0, 10, 15 and 20% dietary levels. The higher values recorded in this study could perhaps be due to differences in breed, age, management system among others (Shanawany, 1988) much as birds used in this study were kept in battery cages and fed different test materials.

The average egg weight did not follow any particular trend in relation to the test diets. Values recorded were slightly lower than the 58g recorded by Oluyemi and Roberts (1979). Longe and Adekoya (1988) also reported similar values (55.4, 57.0, 57.6, 56.9 and 53.9g) for average egg weight when palm kernel meal was iso-nitrogenously substituted at 15.9 and 31.8% into a maize – groundnut meal based diet and fed to 26 week old Ross Brown pullets for 12 weeks.

Egg quality characteristics: According to Card and Nesheim (1975), yolk index is a measure of the standing up quality of the yolk; yolk value for a normal fresh egg should range between 0.40 and 0.42. This is below the range obtained in this study (0.51 to 0.54). This can be explained with the observation of Card and Nesheim (1975) who reported that the holding conditions during storage and more importantly the genetic conditions in the hen rather than the quality of the diet have the most influence on the yolk index and Haugh units. The yolk indices obtained in this work are also within the normal range of 0.3 to 0.5 as stated by Romanoff and Romanoff (1949).

Egg shell thickness was not significantly influenced by the dietary treatments and was lower than the values reported by Oluyemi and Roberts (1979), who reported an average of 0.35mm for the humid tropics. The differences in the values might not be unconnected with breed type and the number of eggs produced during the period. The breeds available now are quite different in the higher number of eggs they can produce compared with those in the late 70's. In addition, feeding is one of the factors responsible for good quality shells. According to Stadelman (1977) egg shell thickness is one of the most direct measures of determining shell quality. Since the shell thickness did not decrease as CSC inclusion increased it indicates that the quality was not affected by CSC inclusion.

Haugh unit is a measure of albumin quality. And in this study it was not affected by dietary treatment. The Haugh unit values obtained from this study were similar to values reported by Oluyemi and Roberts (1979) and Longe and Adekoya. (1988). According to Card and Nesheim (1975), deterioration of eggs affects the yolk and albumen contents. The deterioration may occur in the form of shrinkage, liquefaction and gaseous exchange. Shrinkage involves the evaporation of moisture from the yolk and albumen contents of an egg. The numerous pores in eggs would make the albumen to lose more moisture. According to Oluyemi and Roberts (1979), egg shrinkage makes the albumen to become watery. Egg liquefaction reduces the value of the albumen as water passes from it to the yolk. This leads to lowered viscosity of the albumen white, Card and Nesheim (1975).

Excessive gaseous exchange through the numerous pores in poorly formed eggs has been reported to reduce haugh units (Oluyemi and Roberts, 1979). In this study this shortcomings were not observed given an indication that CSC based diets did not have an adverse effect on haugh unit.

Egg shape index is a measure of the conformity of the shape of the egg to the oval shape of the chicken egg. The egg shape index obtained in this study is similar to values reported by Olorede (1998) who fed shea butter cake at 10% and 20% inclusion levels to layers for 10 weeks, the result also agrees with the findings of Bamgbose (1988) who fed various oil seed cakes to layers.

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Effect of Seafoods (Periwinkle, Bonkafish and Crayfish) and Vegetable Oils Enriched Meal on Cardiovascular Disease

J.I. Ndem, M.I. Akpanabiatu* and E.U. Essien
Department of Biochemistry, Faculty of Basic Medical Sciences,
University of Calabar, P. M. B. 1115, Calabar, Nigeria

Abstract: Periwinkle (Tympanotonus fustcatus), Crayfish (cambarellus diminutus) and Bonka fish (Ethimalosa fimbriata) are local marine food sources of omega-3 fatty acid. Groundnut oil, corn oil and soybean oil are notably high in omega-6 fatty acids. The present study compared changes in haematological and biochemical indices in rats fed with local marine foods (periwinkle, bonka fish and crayfish) and vegetable oils (groundnut, soybean and corn oil) enriched meals. Rats in all the experimental groups had a significant ($P< 0.05$) increase in the Hb, PCV and RBC values and a non-significant decrease ($P> 0.05$) in the WBC counts, when compared with the control. The results of the lipid profile of the test groups on omega-3 and omega-6 enriched pellets were significantly lower than that of control but the HDL-C concentrations were significantly higher in these groups. Similarly rats on pellets enriched with local marine foods (periwinkle, bonka fish and crayfish) considered to be rich in omega-3 fatty acid had significant decreased ($P < 0.05$) cholesterol, and HDL – C concentrations while TG, VLDL and LDL-C increased significantly when compared with control. These results suggest that consumption of diet enriched with periwinkle, bonka fish, crayfish and oil rich in omega-3 and omega-6 polyunsaturated fatty acids may prevent cardiovascular disease. This may be one mayor reason for low incident of coronary heart disease among the poor rural people that consumed basically periwinkle, bonka fish and cray fish as their main sources of protein

Key words: Periwinkle, bonka fish, crayfish, groundnut oil, corn oil, soybean oil, cardiovascular disease

Introduction

Human diet is a key player in the development of degenerative human disease such as cardiovascular disease and the mechanism is multifaceted. Today, human attention is turn to the consumption of natural food, sea food products and food derived antioxidants such as vitamins, as a means of freeing our world of the devastating effect of degenerative diseases. Consumption of food rich in saturated fats have been associated with degenerative diseases such as coronary heart disease, cancer and cerebra-vascular disease (Renaud and Lorgeril, 1992; Stephen and Sieber, 1994; Lapinskas, 2001).

An important fact is that we need both omega-3 and omega-6 polyunsaturated fatty acids for normal functioning of the body but most people consume far more of one type than the other (Rudin and Clara, 1996). This is because westernized dietary pattern seem to favor the consumption of more omega-6 than omega-3 fatty acids. The main reason for this deluge is the growing reliance on vegetable oil such as corn, safflower, sunflower, groundnut and cotton seed oils (Willet, 1994). This imbalance has therefore resulted in high rate of heart disease, cancer, obesity, autoimmune disease, allergies, diabetes and depression (Werbach, 1993; Shils *et al*., 1999). In Nigeria, especially among the affluent class, congestive heart failure, hypertension and other degenerative disease may be attributed to reduced intake of balanced ratio of omega-3 and omega-6 fatty acids enriched diet. It is well known that Eskimos eating their traditional marine diet have no record of heart disease compared to other nationalities. They also have lower levels of rheumatoid arthritis and myocardial infarction (Linder, 1992). Their cardio protective effects have been reported to be due to their high consumption of PUFA (Christensen *et al*., 1997). It has also been reported that for maximum benefit from the two kind of essential fatty acid a ratio of 1:1 is required (Hass, 1992). This is however contrary to the report that most people consumed 20g to 30g of omega-6 to every 1g of omega-3 (Simopoulos, 1999).

Our goal is to use locally available sea food rich in omega-3 and oils rich in omega-6 essential fatty acids to feed experimental animals and analyze their lipid profile as an index of cardio-protective effect of these meals. The haematological indices are also assay to assess the health status of the experimental animals, couple with their immunological state.

Materials and Methods

Animals/feed materials: Twenty-eight adult male and female albino Wistar rats were obtained from the Animal house of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar

Table 1: Diet formulation and composition

Components	GRP I (control) (g)	GRP II (g)	GRP III (g)	GRP IV (g)
Grower Mash	4200	3360	3360	3360
Periwinkle	-	280	140	-
Bonka Fish	-	280	140	-
Cray Fish	-	280	140	-
Groundnut oil	-	-	420	-
Corn Oil	-	-	-	420
Soy Bean Oil	-	-	-	420
Total (g)	4200	4200	4200	4200

for this study. The animals weighed between 260 – 280 grams each. All the animals were kept in plastic cages with stainless mesh at the bottom to prevent faeces and feed dropping from mixing with the experimental animal feed. The experimental animals were divided into four groups of seven animals each (four males and three females). The males were kept in separate cages from the female to prevent mating during the course of the experiment. The rat pellets was obtained from Bendel feed and flour mill limited Benin City, Nigeria. Crayfish, periwinkle and bonka fish were purchased from Uyo Main Market, Uyo, Akwa Ibom State, Nigeria. The edible portions of the periwinkle were removed from the shell and dried in the oven (Plus II Gallankamp) at 50°C overnight. The dry periwinkle, crayfish and bonka fish were individually ground into powder form with the use of an electric blender (National blender) and store in polyethylene sac. Groundnut oil, corn oil and soybean oil were obtained off counter from Uyo Main Market in Uyo, Akwa Ibom State, Nigeria. The oils were manufactured by Grand Cereals and Oil Mills Limited, Jos, Nigeria.

Experimental design and diet formulation: The experimental animals were randomly assigned into four groups. Group 1 animals served as the control and were fed with rat pellets only (Diet I). Group II animals were fed with rat pellets enriched with seafoods highly rich in omega-3 fatty acids (*cambarellus diminutus*, *Tympanotonus fuscatus* and *Ethmalosa fembriata*) (Diet II). In Group III, an attempt was made to balance omega-3 and omega-6 fatty acids by combining the seafoods (crayfish, periwinkle and bonka fish) with groundnut oil in equal proportions (Diet III). Group IV animals were fed pellets enriched with corn oil and soybean oil as good sources of omega-6 fatty acid (Diet IV). The different diets were prepared in the ratio of 1:4 as represented in Table 1. All the experimental animals were fed *ad libitum* for five weeks and they had free access to drinking water.

Collection of blood: At the end of the experimental period all the animals were anaesthetized using chloroform. The animals were dissected and blood samples were obtained by cardiac puncture into EDTA tubes for haematological indices determination and heparinized sample tube for plasma preparation. Plasma samples were obtained from whole blood by centrifugation at 3000 g for 10 minutes, using a bench top centrifuge (MSE, England) and stored in the refrigerator at 4°C. Haematological indices and Lipid profile determinations were carried out within 24 hours of sample collection.

Biochemical and haematological determinations: Plasma lipid profile was determined using standard reagent kits obtained from Randox Laboratory UK. Serum total cholesterol determination was according to the enzymatic colorimetric endpoint methods (Richmond, 1972; Roeschlau *et al.*, 1974; Trinder, 1969). HDL-cholesterol was measured by combination of the methods (Trinder, 1969; Lopes-Virella *et al.*, 1977). LDL-cholesterol was obtained by calculation using the formula provided in Randox HDL-cholesterol kit booklet. Plasma TG was assayed by colorimetric methods (Trinder, 1969; Tietz, 1990). VLDL and LDL were obtained by calculation according to method in the HDL-cholesterol kit manual (Randox Kit)

The percent packed cell volume (% PCV), white blood and Red blood cells count were determined by method of Dacie and Lewis, 1975. Haemoglobin was measured using the method of Alexander and Griffith, 1996

Statistical analysis: Data are expressed as mean ± SD. Comparisons of data were by the student's t-test and $P \leq 0.05$ was considered significant.

Results

The haematological indices of experimental animals are as shown in Table 2. The results showed that the haemoglobin concentration increases with increase in the content of essential fatty acid in the supplement although when the test groups (II, III and IV) were compared with the control group 1; there was no significant change ($P \leq 0.05$). Animals on diet enriched with omega-3 and omega-6 fatty acids (group III) had the highest value of % PCV, which was significantly different from the control ($P \leq 0.05$) and other groups. Animals in group II and IV, which were fed omega-3 and omega-6 enriched rat pellets respectively, had significantly lower % PCV than the control group ($P \leq 0.05$). The RBC

Table 2: Haematological indices of adult albino Wistar rats fed with different ratios of n-3 and n-6 polyunsaturated fatty acids supplemented diets

Parameters+/Groups	Hb(g/dl)	PCV (%)	RBC ($x10^{12}$/l)	WBC ($x10^{9}$/l)
Group I(Control)	9.26 ± 1.60	47.63 ± 0.48	1.945 ± 0.053	4.43 ± 0.10
Group II	10.32 ± 0.42	45.25 ± 0.96	2.140 ± 0.054*	5.25 ± 0.13
Group III	12.23 ± 0.29	50.75 ± 0.96*	2.385 ± 0.081*	5.58 ± 0.17
Group IV	11.29 ± 0.28	43.00 ± 0.81*	2.287 ± 0.084*	5.35 ± 0.13

+ = Mean ± SD. * Significantly different from the control value ($P<0.05$)

counts for the experimental groups II, III, IV showed a significant change ($P \leq 0.05$) when compared with the control. These results also showed that there were no significant changes ($P \geq 0.05$) in WBC counts when the test groups were compared with control.

Table 3 depicts the plasma lipid profile of Wistar albino rats fed with different ratios of n-3 and n-6 fatty acids enriched pellets. The plasma total cholesterol and triacylglycerol (TG) showed that group III recorded the lowest values with the highest value recorded by the control group I. The lipid profile showed significant change ($P \leq 0.05$) when compared with control and among the groups except in the plasma total cholesterol concentration of group II. Rats fed n-3 and n-6 fatty acids enriched pellets had significantly elevated plasma HDL-cholesterol when compared with the control and when compared with omega-3 and omega-6 enriched pellets, respectively. Plasma VLDL-cholesterol recorded significantly ($P \leq 0.05$) lower concentration for rats fed n-3 and n-6 enriched pellets with rats fed n-6 enriched pellets only recording the highest VLDL value. Group to group comparison also showed significant change ($P \leq 0.05$) in the VLDL values. Experimental animals on diet enriched with the mixture of n-3 and n-6 fatty acids (group III) recorded the lowest value of LDL-cholesterol but were not significant ($P \geq 0.05$) when compared with that of control animals and other groups, excepts when group II was compared with group IV. However, when all the test groups (II, III, and IV) were compared with control group, there were significant changes ($P \leq 0.05$).

Discussion

Essential fatty acids (omega-3 and omega-6) and their derivatives have been known to lower cholesterol levels in the blood, which is an important parameter for reducing the incidence of coronary heart disease (CHD). Marine foods, especially fish have been reported to be rich in n-3 fatty acids while omega-6 fatty acids are found mostly in vegetable oil (Shils *et al.*,1994). Eskimos and Japanese fishermen have been observed to have low incidence of myocardial infarction although they consume a diet high in animal protein, fat and cholesterol (Dyerberg and Jorgensen, 1982). This has been attributed to high levels of omega-3 fatty acid in their diets.

Commonly eaten in Nigeria especially among people in the riverine areas and low income earners are seafoods, which include bonka fish, crayfish and periwinkles. Vegetable oils such as groundnut, corn and soybean oil are commonly eaten by the affluent groups in Nigeria. Essien and others have reported that oils from vegetables are good source of n-3 and n-6 essential fatty acids (Essien *et al.*, 1995). In this study rat pellets supplemented with different ratios of these essential polyunsaturated fatty acids sources were fed to experimental animals in order to determine their effect on the risk of CHD in experimental animals.

Furthermore, supplementing rats pellets with different ratios of omega-3 and omega-6 PUFA which were: omega-3 fatty acid enriched diet, omega-3 and omega-6 fatty acids enriched diet and omega-6 fatty acid enriched diet showed appreciable improvement in the haematological indices as evidenced by increased Hb, PCV and RBC counts and decreases in WBC counts. The significant increases ($P \leq 0.05$) in Hb, PCV and RBC counts and the insignificant decreases in WBC counts suggested that the enriched diets did not induce anaemia in the experimental animals and this was highly exhibited by the omega-3 and omega-6 fatty acids enriched diet. The lipid profile of animals indicated significantly decreased concentrations of total cholesterol, VLDL-C, TG and increased concentration of HDL-C for the omega-3 and omega-6 fatty acids enriched pellets (supplemented diet). These findings suggest the ameliorating effects of this diet on CHD. Animals on Omega-3 enriched pellets (supplemented diet) showed decreased concentrations of total cholesterol and HDL-C with increased concentration of TG, VLDL-C and LDL-C compared with the control. These results however, suggest an atherogenic potential for the omega-3 rich pellet supplements. Also animals on omega-6 enriched diet showed similar pattern as the animals fed pellets enriched with omega-3 and omega-6 fatty although a higher effect was shown by the later. From the results n-3 and n-6 rich diet is expected to prevent atherogenicity. This is in line with some reports by other authors that a concentrate of free fatty acids of fish oils, prevent ventricular fibrillation and sudden cardiac death in reliable dog model with very high probability (Billman and Hallaq, 1994; Billman *et al.*, 1997).

The results of this investigation suggest that consumption of a diet enriched with both omega-3 and omega-6 would be of more health benefit and may help prevent coronary heart disease. The same tendency but in a lesser extent was exhibited by omega-6 enriched

Table 3: Lipid profile of adult albino Wistar rats fed with different ratios of n-3 and n-6 polyunsaturated fatty acids supplemented diets

Parameters+/Groups	Plasma Total Cholesterol	Plasma TG	Plasma HDL-Cholesterol	Plasma VLDL-Cholesterol	Plasma LDL-Cholesterol
Group I (Control)	125.00 ± 3.78*,**	91.25 ± 0.72	27.57 ± 0.30	18.27 ± 0.13	69.69 ± 3.43
Group II	119.94 ± 2.80*	98.09 ± 3.07*,**	29.26 ± 0.59*,**	19.72 ± 0.60*,**	70.76 ± 3.33*,**
Group III	109.50 ± 2.64*,**	85.23 ± 0.94*,**	37.04 ± 0.81*,**	17.06 ± 0.18*,**	64.97 ± 2.60*
Group IV	114.42 ± 1.92*,**	88.92 ± 0.61*,**	30.56 ± 0.68*,**	17.77 ± 0.13*,**	66.18 ± 2.00*,**

+ = Mean ± SD. * Significantly different from the control value (P<0.05). * Significantly different from the corresponding test group (P<0.05)

diet. However, we observed also that consumption of only omega-3 fatty acid enriched diet though nutritionally very beneficial may not be generally protective against coronary heart disease since long term consumption is likely to increase the risk for coronary heart disease.

In conclusion, we are confident to suggest that the rural Africans, especially those of the coastal regions that consumed mostly the types of fishes/crayfish used in this experiment have good reason for having low incidence of coronary heart diseases. This beneficial health effects are attributed to the supplementation of animal feed with rich sources of n-3 and n-6 fatty acids in the right ratios. Also the nutritional quality of these sea foods may be a remedy to coronary heart diseases, hence the need for African health workers to promote the consumption of marine foods as a strategy of managing CDH, a disease that is alien to the African culture.

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Evaluation of Enzyme (Maxigrain®) Supplementation of Graded Levels of Palm Kernel Meal (PKM) on the Performance of Broiler Chickens

*P.M. Esuga, A.A. Sekoni, J.J. Omage and G.S. Bawa
Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria

Abstract: This study was conducted to evaluate the effects of Palm Kernel Meal (PKM) in diets supplemented with or without enzyme (Maxigarin®) as replacement for Maize in broiler diets. Four hundred and five day old Arbor acres broiler chickens were randomly allotted to nine isonitrogeneous diet where PKM was included in the diet at 10, 20, 30, and 40% levels. Four of the diets contain PKM without Maxigrain® while the other four contained PKM with Maxigrain® supplementation. The Maxigrain® was added to the already formulated diet (supplementation) at 0.01% to four of the nine diets. At the starter phase the final body weight, weight gain and average daily weight gain were significantly ($P<0.001$) higher in 10% and 20% PKM diets Maxigrain® supplementation compared to other treatments. Feed intake was significantly ($P<0.001$) higher in the control, 10% and 20% PKM diets with Maxigrain®. The feed : gain ratio was significantly ($P<0.001$) lower in the 10% PKM diet with Maxigrain® compared to all other treatments. All levels of PKM diets with Maxigrain® were significantly ($P<0.001$) lower than the corresponding levels without Maxigrain®. The feed cost/kg weight gain were significantly ($P<0.001$) lower in all PKM diets with and without Maxigrain® compared to the control. At the finisher phase, the final weight, weight gain and average daily weight gain were significantly ($P<0.001$) higher in the 10% and 20% PKM diets with Maxigrain® compared to all other treatments. Feed intake was significantly ($P<0.001$) higher in all PKM diets with and without Maxigrain® compared with the control. Feed : gain ratio and feed cost/kg weight gain (N) were significantly ($P<0.001$) lower in the control and all PKM diets with Maxigrain® supplementation compared to all PKM diets without Maxigrain®. The results indicate that Maxigrain® supplementation of PKM diets improved the utilization of PKM. Diets with 10 and 20% inclusion of PKM and Maxigrain® were better than the control maize based diets. The dressed weight, neck, liver, lungs, kidney, abdominal fat, pancrease, spleen and length of intestines were significantly ($P<0.001$) different across treatments. Similarly, the percentage weight of the breast, thigh, heart and the intestines were significantly ($P<0.001$) different across treatments with no particular trend established. The drumstick, wings, head and gizzard were significantly ($P<0.05$) different across treatments. No significant difference in the dressing percentage and the back across the treatments.

Key words: PKM, Maxigrain®, supplementation, broiler performance

Introduction

The advent and use of commercial feed enzymes in livestock feeding has opened new horizon for the use of hitherto waste feedstuff. Results of experiments (Atteh, 2001) showed that with the use of a bacterial xylanase enzyme, it was possible to replace 50% of maize with wheat offal without detrimental effect on broiler performance. Atteh (2001) reported 56% and 26% increase in apparent metabolizable energy (AME) of wheat offal and BDG respectively when supplemented with xylanase enzyme. Palm Kernel Meal is one of the abundant agricultural by-products, is cheap and readily available. It is aflatoxin free, palatable and has considerable potential as a carbohydrate and protein source. However, due to it's low nutritive value, grittiness and potential for deterioration in unhygienic conditions, a large amount of PKM is often discarded and can create environmental problems in the future (Sundu and Dingle, 2005). With the availability of feed enzymes to digest fibre, there is need to evaluate these feeding value of enzyme treated PKM. From the composition of PKM, it appears that three main enzymes are needed to improve their nutritive value namely: mannanase, β-galactosidase and cellulase. (Balasubramaniam, 1976). PKM has been reported to contain mannan and galactomannan and these are likely to have anti-nutritive properties. Of the total NSPs in PKM, 78% is mannan, 3% is arabinoxylans, 3% is glucoronoxylans which were found to be water-insoluble and 12% is cellulose (Dusterhoft *et al*., 1992). These problems are mostly tackled by either a careful formulation of diet for protein and energy balance or the inclusion of enzymes, particularly mannanase, galactosidase and cellulase. The role of enzymes as feed additive in poultry diets is well established. Hastings (1946) and Allen *et al*. (1997) all observed that enzyme addition to monogastric animal feed reduced viscosity of ingesta in the intestine and showed a marked improvement on the various morphological effects of feeding fibrous materials to non-ruminant animals. The use of PKM in poultry diets

has been practiced for several decades. Its low level of key essential amino acids (Lysine and Methionione in particular), high dietary fibre (particularly in the form of β-mannan) and grittiness have precluded its inclusion in broiler diets. Enzymes have been approved for use in poultry feed because they are natural products of fermentation and therefore pose no threat to the animal or the consumer. (Vukic Vranjes and Wenk, 1993) Their use in poultry feeds has predominantly been related to the hydrolysis of fibre or non-starch polysaccharide (NSP) fraction of cereal grains. These NSPs cannot be digested by the endogenous enzymes of poultry and can have anti-nutritive effects. They cause an increase in viscosity of intestinal content and entrap large amounts of well digestible nutrients like starch and proteins. This leads to an impaired digestion and digestive problems. (Almirall *et al.*, 1995)

This study aims to determine the effects of Maxigrain® supplementation of Palm Kernel Meal on the performance of broiler chicks, evaluate the economics of raising broiler chicks on Maxigrain® supplemented Palm Kernel Meal, and determine the effects of Maxigrain® supplementation of Palm Kernel Meal on carcass characteristics of broilers.

Materials and Methods

The starter phase lasted from 0-4 Weeks. Four hundred and five day old chickens (mixed sexes) of Arbor acres strain were used for this study. The birds were weighed at day old and randomly assigned to nine isonitrogeneous diets, formulated at 23% crude protein as shown on Table 2. Here the compounded diet was supplemented with Maxigrain® at 100gm per metric ton inclusion irrespective of the PKM levels in the diet. Chemical analysis of diets were done by the method of A.O.A.C. (1990).

The birds were housed on deep litter system in a 4x2+1 factorial combination of dietary levels of PKM with or without Maxigrain® in a Completely Randomized Design (CRD). The treatments were replicated three times with fifteen chicks per replicate. The chicks were brooded conventionally in a deep litter system. Feed and water were supplied *ad-libitum* while vaccinations were administered appropriately. Mortality was recorded as it occurred. The birds were weighed weekly, live weight, weight gain, average daily gain, feed intake, water intake, feed : gain ratio and feed cost (N/kg gain) were determined at weekly intervals.

The finisher phase lasted from 5-8 weeks of age. At the end of the starter phase, the birds were fed on the finisher diet at the beginning of the fifth week. There were nine(9) diets, the composition of which is shown in Table 3. The diets were isonitrogeneous at 20% crude protein. Each treatment was replicated three times. Feed and water were supplied *ad-libitum* during the experimental period. Mortality was recorded as it occurred. Weekly records of live weight, weight gain, average daily gain, feed intake, water intake, feed : gain ratio and feed cost (N/kg gain) were determined. At the end of the eight week of feeding trial, two birds from each replicate were randomly selected, weighed for carcass evaluation.

All data obtained were subjected to the analysis of variance using the General Linear Model procedure of Statistical Analysis System (SAS) computer software package (1985). Significance of difference between means was determined by applying the Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

Results

Effects of Maxigrain® supplementation of PKM on broiler performance: The effect of Maxigrain® supplementation on the performance of broiler starter chicks (0-4 weeks) is presented in Table 4. The final weights and average daily gain of the 10 and 20% diets with Maxigrain® were significantly ($P<0.001$) superior to control while 20, 30 and 40% diets without Maxigrain® were inferior to control. The control diet was similar to 30 and 40% diets with Maxigrain® and 10% diet without Maxigrain® in their final weights. All the Maxigrain® supplemented diets show relatively higher weights than diets without Maxigrain® at comparative levels.

Feed intake was similar between control and 10% Maxigrain® supplemented diet but significantly ($P<0.001$) different compared with all other diets with or without Maxigrain® supplementation. The feed in take among Maxigrain® supplemented diets were relatively higher than diets without supplementation. The feed in take at 40% PKM inclusion with or without Maxigrain® was similar.

Feed : gain ratio was similar between control and all other diets with or without Maxigrain® except 10, and 20% diets with Maxigrain® which were superior to control. The best diet was 10% with Maxigrain® supplementation with a feed : gain ratio value of 1.34. The feed : gain ratio at 30%, were similar with or without Maxigrain®.

Feed cost/kg weight gain was significantly ($P<0.05$) different between control (with the highest value) and all other diets with or without Maxigrain® supplementation. The feed cost/kg weight at 10, 20 and 30% with Maxigrain® is similar to 10, 20, 30 and 40% diets without Maxigrain®. The best diet in feed cost/kg weight was at 40% with Maxigrain® at a value of N50.43.

Water intake shows that the control was similar to 10% diet with Maxigrain® but significantly ($P<0.001$) higher than all other diets.

The water intake at 10, 20, 30% PKM inclusion level in the Maxigrain® supplemented diets were significantly ($P<0.001$) higher than in diets without Maxigrain® supplementation. The water intake at 40% PKM inclusion was similar in diets with or without Maxigrain®.

Table 1: Endogeneous enzymes in poultry

Organ	Enzyme produced	Substrate acted upon	End product
Mouth (Saliva)	Alpha-Amylase	Starch	Glucose, Maltose Dextrins
Proventiculus	Pepsin	Protein	Peptides
Pancrease	Amylase	Starch	Glucose, Maltose Limit Dextrins. Fatty Acids, Monoglycerides.	Amino acids and small peptides.
	Lipase	Fat	Amino acids
	Trypsin	Proteins	
	Chymotrysin	Peptides	
	Elastase		
	Carboxy peptidase		
Intestinal Mucosa	Oligo-1,6 - glucosidase	Dextrin	Glucose
	Maltase		
	Sucrase	Maltose	Glucose
	Amino-petidase	Sucrose	Glucose & Fructose
	Dipeptidases	Peptides	Amino acids
		Dipeptides	Amino acids

Source: Card and Neshien (1972)

Table 2: Percentage composition of starter diets

Ingredient	Control	10%	20%	30%	40%
Maize	55.00	47.50	40.00	33.00	25.00
Soyabean Meal	40.00	37.50	35.00	32.00	30.00
*PKM	0.00	10.00	20.00	30.00	40.00
Bone meal	2.60	2.60	2.60	2.50	2.60
Limestone	1.60	1.60	1.60	1.60	1.60
Salt	0.35	0.35	0.35	0.35	0.35
**Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Cost/kg diet(N)	43.00	40.00	38.00	35.00	33.00
Calculated analysis of starter diets					
ME Kcal/kg	2800.00	2757.00	2657.00	2561.00	2456.00
Crude Protein(%)	23.00	23.00	23.00	23.00	23.00
Crude Fibre(%)	4.00	5.00	5.40	6.00	7.00
Ether Extract(%)	3.50	3.80	4.00	4.20	4.50
Lysine (%)	1.35	1.33	1.30	1.30	1.30
Methionine(%)	0.44	0.45	0.46	0.48	0.50
Calcium (%)	1.50	1.50	1.50	1.50	1.50
Available Phosphorus(%)	0.70	0.70	0.70	0.70	0.70

**Bio-mix premix supplied per kg of diet: Vitamin A 12500 I.U Vit D_3 2500 I.U Vit E 50mgL Vit K_3 2.5mg; Vit B_1, 3.0mg: Vit B_2 6.0mg; Vit B_6 6.0mg: Niacin 40.0mg; calcium pantothenate 10.0mg; Biotin 0.80mg; Vit B_{12} 0.25mg; Folic acid 1.0mg; Chloline chloride 300mg; manganese 100mg: Iron 50mg; Zinc 45; copper 2.0mg; cobalt 0.25mg; iodine 1.55, selenium 0.1mg.

The water : feed ratio was similar in all diets with or without Maxigrain®.

There was no significant ($P>0.05$) difference in mortality in all diets with or without Maxigrain® supplementation.

Table 5 : shows the dietary effects of the treatments on the performance of broiler finisher chickens (5-8weeks). The initial weight of the birds in this phase is the same as the final weight of the starter phase of this study.

Control was significantly ($P<0.001$) different compared with all other diets with or without Maxigrain® supplementation in the final weights but inferior to 10 and 20% diets with Maxigrain®. All the supplemented diets were significantly ($P<0.001$) higher in their final weights when compared with the unsupplemented diets at the same level of PKM inclusion. PKM inclusion at 10% was the best followed by 20% in the supplemented diets in their final weight gain while the control diet was significantly ($P<0.001$) superior to all the other diets with or without Maxigrain®. The supplemented diets were significantly ($P<0.001$) different at comparable levels of inclusion with the unsupplemented diets.

The feed intake by the control was the lowest and significantly ($P<0.001$) different from all other diets with or without Maxigrain® supplementation. Feed intake increased with increasing level of PKM inclusion with or without Maxigrain® supplementation. Maxigrain® supplemented diets at 30 and 40% were similar when compared with the same levels without supplementation.

The feed : gain ratio between control, 10 and 20% Maxigrain® supplemented diets were similar. The feed : gain ratio increased with increasing levels of PKM with

Table 3: Percentage composition of finisher diets

Ingredient	Control	10%	20%	30%	40%
Maize	65.00	55.00	50.00	40.00	32.00
Soyabean Meal	30.00	30.00	25.00	25.00	23.00
*PKM	-	10.00	20.00	30.00	40.00
Bone meal	2.60	2.60	2.60	2.60	2.60
Limestone	1.60	1.60	1.60	1.60	1.60
Salt	0.35	0.35	0.35	0.35	0.35
**Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Cost/kg diet(N)	40.00	38.50	35.00	33.00	31.00
Calculated analysis					
ME Kcal/kg	3000.00	2850.00	2758.00	2635.00	2528.00
Crude Protein (%)	20.00	20.00	20.00	20.00	20.00
Crude Fibre (%)	3.00	4.00	5.00	6.00	7.00
Ether Extract (%)	3.70	3.90	4.10	4.30	4.50
Lysine (%)	1.10	1.14	1.00	1.00	1.00
Methionine (%)	0.39	0.42	0.42	0.40	0.45
Calcium (%)	1.50	1.50	1.50	1.50	1.50
available Phosphorus (%)	0.60	0.60	0.60	0.60	0.60

**Bio-mix premix supplied per kg of diet: Vitamin A 12500 I.U: Vit D_3 2000 I.U: Vit E 30mg; Vit K_3 3.75mg; Vit B_1, 2.5mg: Vit B_2 5.0mg; Vit B_6 3.75mg: Niacin 30mg; Pantothenate acid 12.5mg; Biotin 0.50mg; Vit B_{12} 25mg; Folic acid 0.750mg; Chloline chloride 375mg; Manganese 150mg: Iron 25mg; Zinc 37.5; copper 2.0mg; Iodine 1.0mg; cobalt 0.200mg; selenium 0.100mg; Growth promotant 20mg; Antioxidant 100mg. *The PKM based diets consists of 10%, 20%, 30% and 40% with Maxigrain® and 10%, 20%, 30% and 40% without Maxigrain®. · One Naira (N) is equivalent to 128 US Dollars ($)

or without Maxigrain® supplementation. The best diet was 10% with Maxigrain® at a value of 2.26 when compared with all other diets with or without supplementation.

Feed cost/kg gain was observed to have similar trend as in the feed : gain ratio at all levels. The best diet was 10% with supplementation at a value of N93.46 similar to control and 20% diet with supplementation.

There was significant ($P<0.001$) difference in water intake between control and all other treatments with or without supplementation. There was significant ($P<0.001$) difference at 10% PKM inclusion with Maxigrain® compared with the same level without Maxigrain®. Water intake was similar at 20, 30 and 40% diets with Maxigrain® supplementation were similar when compared with the same levels without Maxigrain®.

There was no significant ($P>0.05$) difference in mortality in all the treatments with or without Maxigrain® supplementation.

Water : feed ratio was calculated without any observable adverse effect of increasing levels of PKM with or without Maxigrain on water consumption ratio.

Table 6 show the results of the carcass analysis of the birds fed the Maxigrain® supplemented diets. The results shows that there was significant ($P<0.001$) difference in the dressed weight of the control diet compared to all other diets with or without Maxigrain® supplementation but was observed to be significantly ($P<0.001$) inferior to 10 and 20% diets with Maxigrain®. The dressed weight was progressively lower with increasing levels of PKM with or without supplementation except at 10 and 20% Maxigrain® supplementation where dressed weight was higher.

There was no significant difference ($P>0.05$) in the dressing percentage and the back of the birds with or without supplementation. There was significant difference ($P<0.05$) in the breast, neck, thigh, drumstick, wings, head, lungs, gizzard, pancrease, intestines, spleen and intestinal length but there was no specific trend established.

The %heart of the birds fed supplemented diets were significantly ($P<0.001$) different than and those fed the unsupplemented diets which were bigger than control and all supplemented diets. The weight of the heart in the control diet was similar to the weight of the heart of all Maxigrain® supplemented diets at all levels of inclusion.

The %liver in the control was similar to 10 and 20% diets with Maxigrain® and 10% without Maxigrain® At all levels of inclusion, the liver in Maxigrain® supplemented diet were significantly ($P<0.001$) lower than those in the unsupplemented diets where the size of the liver was observed to increase with increasing levels of PKM inclusion with or without Maxigrain®.

The %kidney was similar between control, 10 20 and 40% diets with Maxigrain® and 10% without Maxigrain® but significantly ($P<0.001$) lower than the other treatments. The size of the kidney was observed to increase with increasing levels of PKM in the unsupplemented diets compared with the Maxigrain® supplemented diets. The abdominal fat was significantly

Table 4: Performance of broiler starter (0 - 4 weeks) chicks on PKM diets with or without Maxigrain® supplementation

Parameters	control	PKM 10+	With 20+	Maxi grain® 30+	Diets 40+	PKM 10-	without 20-	Maxi-grain® 30-	diets 40-	SEM	Levels of Signi-ficance
Initial weight(g)	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	-	NS
Final weight(g)	916.33^{c}	1034.67^{a}	978.00^{b}	895.67^{c}	878.33^{c}	894.00^{c}	826.00^{d}	748.33^{e}	745.67^{e}	8.30	***
Weight gain(g)	876.33^{c}	994.67^{a}	938.00^{b}	855.67^{c}	838.33^{c}	854.00^{c}	786.00^{d}	708.33^{e}	705.67^{e}	8.30	***
Ave Daily gain(g)	31.30^{c}	35.52^{a}	33.50^{b}	30.56^{c}	29.94^{c}	30.50^{c}	28.07^{d}	25.30^{e}	25.20^{e}	0.29	***
Feed in take (g)	1363.67^{a}	1332.67^{ab}	1311.33^{b}	1243.00^{c}	1181.00^{d}	1248.00^{c}	1198.00^{d}	1171.00^{d}	1168.00^{d}	8.47	***
Feed:Gain Ratio	1.56^{de}	1.34^{a}	1.40^{bc}	1.45^{bcde}	1.41^{bcd}	1.46^{cde}	1.53^{de}	1.66^{e}	1.6^{e}	0.02	***
FeedCost/kgwt(N)	66.92^{c}	57.35^{b}	57.06^{b}	54.92^{b}	50.43^{a}	58.46^{b}	58.02^{b}	57.94^{b}	54.77^{b}	0.75	*
Water intake (lts)	3206.33^{a}	3209.33^{a}	3086.33^{b}	2952.00^{c}	2787.33^{de}	2834.33^{d}	2828.33^{d}	2756.00^{e}	2756.67^{e}	10.57	***
Water:Feed Ratio	2.36	2.41	2.36	2.38	2.36	2.28	2.36	2.36	2.36		
Mortality	0.33	0.00	0.00	0.00	0.33	0.00	0.00	0.33	0.33	0.12	NS

•abcdef Means with different superscript within the same row are significantly (P<0.001)different

*** = (P<0.001), NS: No significant difference (P>0.05). One Naira (N) is equivalent to 128 US Dollars ($)

(P<0.001) higher in the control group compared to all other treatments. Similarly, the abdominal fat in the Maxigrain® supplemented diets were significantly (P<0.001) lower than the unsupplemented diets at all levels of PKM inclusion.

Discussion

The performance of broiler starter (0-4weeks) chicks with or without Maxigrain® supplementation showed increase in final weight gain, improved feed : gain ratio in the control and Maxigrain®treated diets relative to diets without Maxigrain®. This is expected as Choct (2006) reported the degradation of β - mannan and 70% NSPs into soluble metabolizable products for monogastrics when enzymes are added to high fibre monogastric diets. Feed and water intake was slightly higher up to an optimum of 30% in the Maxigrain® supplemented diets compared to diets without Maxigrain®. Onwudike, (1986), Ezieshi and Olomu (2004) reported higher fed intake of birds fed a PKM based diet compared with a maize – based diet due probably to its faster passage rate in the digestive tract. Feed : gain ratio was better among Maxigrain® supplemented diets compared with the control and all other diets without Maxigrain®. This is similar to the report of Atteh (2000) who observed an improvement in weight gain and feed conversion efficiency in birds fed enzyme supplemented diets. Ariff Omer *et al.* (1998) and McDonald *et al.* (1995) observed lower weights of birds fed increasing levels of PKM without supplementation.

The performance of broiler finisher (5-8weeks) chicks with or without Maxigrain® indicated that the birds on control and Maxigrain® treated diets had a higher final weight gain, improved feed : gain ratio compared to diets without Maxigrain®. Feed and water intake increased with increasing inclusion of PKM probably due to the critical need for energy in finisher chickens but this did not translate into higher live weight or weight gain especially among diets without supplementation. Feed : gain ratio and feed cost/kg weight gain was best at 10% diet with supplementation similar to the control. This is supported by the report of Classen *et al.* (1995) and Scott *et al.* (1998) who reported that the responses to the use of enzymes is highest on poor quality raw materials. Panigrahi and Powell (1991) found that the inclusion of PKM up to 50% was tolerated provided that the birds were kept to 7 weeks of age. The water intake of birds fed PKM based diets is also increased (Panigrahi and Powell, 1991) and there is increased moisture content of excreta (Onifade and Babatunde, 1998).

The economic analysis reveal a comparative feed cost/kg weight (N/kg) similar to the control diet in the Maxigrain® supplemented diets up to 20% inclusion of PKM with the best result of N93.46 recorded at 10% PKM inclusion with supplementation. This is expected as the cost per metric ton of maize is widely higher than PKM at about N40.000 to N8,000 for maize and PKM, respectively. Akpodiete *et al.* (2006) reported that the cost of a kilogram feed reduction in cost with increasing PKM inclusion with the lowest cost at the highest PKM replacement level.

The carcass evaluation of broiler chicks fed diets with or without Maxigrain® supplementation showed similar trend as that of the growth performance. The birds fed on Maxigrain® supplemented diets had higher pre-slaughter weight, and carcass weight, compared to those on similar diets without Maxigrain® supplementation. There was no significant variation in the dressing percentage of the birds with or without Maxigrain®.

There was significant variation in the breast, neck, thigh, drum stick, wings, head, gizzard, and intestinal length but no trend was established. The lungs, liver, kidneys, pancrease and intestines were within the normal range reported for chickens suggesting the absence of toxic effects by the treatments.

However, a trend was observed in the heart, liver, kidney and abdominal fat. The heart appears to increase in diets without Maxigrain® supplementation probably due to increased function. This agrees with Brenes *et al.*, (1993), who reported that enzyme supplementation affects the relative size of some organs with relative reductions in the weights of such organs. Similar trend

Table 5: Performance of broiler finisher (5-8weeks) chicks on PKM diets with or without Maxigrain® supplementation

Parameters	Control	PKM 10+	with 20+	Maxi grain® 30+	diets 40+	PKM 10-	without 20-	Maxi grain® 30-	diets 40-	SEM	Levels of Signi-ficance
Initial weight(g)	916.33^{c}	1034.67^{a}	978.00^{b}	895.67^{c}	878.33^{c}	894.00^{c}	826.00^{d}	748.33^{e}	745.67^{e}	8.30	***
Final weight(g)	2107.00^{c}	2376.33^{a}	2279.00^{b}	1870.00^{e}	1722.00^{f}	1942.33^{d}	1654.67^{g}	1377.00^{h}	1304.67^{i}	12.43	***
Weight gain(g)	1190.67^{b}	1341.67^{a}	1301.00^{a}	974.33^{d}	843.67^{e}	1048.33^{c}	828.67^{e}	628.67^{f}	559.00^{g}	9.67	***
AveDaily gain(g)	42.52^{b}	47.92^{a}	46.46^{a}	34.80^{d}	30.13^{e}	37.44^{c}	29.60^{e}	22.45^{f}	19.96^{g}	0.34	***
Feed intake (g)	2781.33^{a}	3036.33^{c}	3259.67^{e}	3269.00^{e}	3397.97^{f}	2923.67^{b}	3130.67^{d}	3263.00^{e}	3388.00^{f}	12.16	***
Feed: Gain Ratio	2.34^{a}	2.26^{a}	2.60^{ab}	3.36^{c}	4.03^{d}	2.79^{b}	3.78^{d}	5.19^{e}	6.10^{f}	0.07	***
FeedCost/kgwt(N)	93.53^{a}	93.46^{a}	94.71^{a}	121.00^{c}	136.13^{d}	107.34^{b}	132.24^{cd}	171.41^{e}	189.18^{f}	2.32	***
Water intake (lts)	6655.67^{a}	7056.33^{b}	7303.67^{d}	7385.67^{e}	7459.33^{f}	7163.33^{c}	7256.00^{d}	7389.33^{e}	7507.00^{f}	10.57	***
Water:Feed Ratio	2.4	2.33	2.24	2.26	2.20	2.45	2.32	2.27	2.22		
Mortality (%)	0.66	1.00	0.33	0.66	0.66	0.33	0.33	0.66	1.00	0.30	NS

•abcdef Means with different superscript within the same row are significantly (P<0.001) different
•*** = (P<0.001) NS: No significant difference (P>0.05).

Table 6: Carcass characteristics of broiler chicks fed on PKM diets with and without Maxigrain® supplementation (expressed as percentage of live weight)

Parameters	Control	PKM 10+	with 20+	Maxi grain® 30+	diets 40+	PKM 10-	Without 20-	Maxi grain® 30-	diets 40-	SEM	Levels of signi-ficance
Live Wt(g)	2073^{b}	2360^{a}	2274^{a}	1875^{c}	1722^{d}	1966^{c}	1641^{d}	1370^{e}	1326^{e}	27.09	***
Dressed Wt(g)	1670^{b}	1871^{a}	1806^{a}	1389^{d}	1353^{ed}	1575^{c}	1273^{e}	1080^{f}	1046^{f}	25.17	***
Dressing %	80.54	79.48	79.42	77.08	78.57	80.06	77.55	78.80	78.80	0.93	NS
Breast%	26^{abc}	29^{a}	25^{bc}	24^{bc}	25^{abc}	23c	28^{ab}	25^{bc}	26^{abc}	0.82	**
Neck%	5.0^{d}	7.7^{ab}	8.0^{a}	7.0^{abc}	5.0^{d}	7.6^{bcd}	5.0^{d}	6.0^{cd}	6.0^{cd}	0.36	***
Thigh%	27.5^{bc}	30.5^{ab}	25.8^{c}	30.7^{a}	25.6^{c}	28.5^{abc}	27^{c}	25.7^{c}	26.6^{c}	0.82	**
Drum stick%	14.9^{a}	13.5^{a}	14^{a}	12.9^{ab}	13a	11^{b}	13.5^{a}	13^{a}	13.5^{a}	0.50	*
Wings%	9.4^{bcd}	9.0^{cd}	8.8^{d}	11^{a}	10^{abcd}	10.5^{abcd}	11^{a}	10.9^{abc}	11^{ab}	0.46	*
Back%	14.0	15.0	13.9	15.3	15.4	14.4	14.4	13.7	14.0	0.59	NS
Head%	2.5b	3.0^{ab}	3.0^{ab}	3.5^{a}	3.0^{a}	2.9^{ab}	2.9^{ab}	3.5^{a}	3.0^{a}	0.16	*
Heart%	0.47^{cd}	0.47^{d}	0.48^{bcd}	0.45^{d}	0.45^{d}	0.5^{abcd}	0.56^{ab}	0.55^{abc}	0.5^{a}	0.03	**
Liver%	1.46^{ef}	1.3^{f}	1.29^{f}	1.86^{cd}	1.99^{c}	1.6^{de}	2.09^{c}	2.4^{b}	2.98^{a}	0.08	***
Lungs%	0.57^{c}	0.58^{c}	0.57^{c}	0.74^{ab}	0.6^{c}	0.55^{c}	0.6^{c}	0.7^{b}	0.8^{a}	0.02	***
Kidney%	0.4^{d}	0.4^{d}	0.44^{cd}	0.5^{bc}	0.45^{cd}	0.4^{d}	0.5^{bc}	0.57^{ab}	0.6^{a}	0.06	***
Gizzard%	2.49^{c}	2.75^{bc}	2.99^{abc}	4.0^{a}	3.8^{abc}	3.0^{abc}	2.66^{bc}	3.6^{abc}	4.0^{ab}	0.37	*
AbdominalFat%	1.5^{a}	0.85^{c}	0.7^{cde}	0.6^{de}	0.5^{e}	1.1^{b}	1.3^{b}	0.8^{cd}	0.6^{de}	0.05	***
Pancrease %	0.32^{c}	0.34^{c}	0.36^{bc}	0.44^{a}	0.49^{a}	0.34^{c}	0.40^{ab}	0.50^{a}	0.40^{ab}	0.02	***
Intestines%	3.0^{c}	4.7^{ab}	4.4^{ab}	4.56^{ab}	4.34^{ab}	3.99^{bc}	4.44^{ab}	5.04^{ab}	5.0^{a}	0.26	**
Spleen%	0.17^{bcd}	0.15^{cd}	0.19^{bc}	0.19^{bc}	0.17^{bcd}	0.12^{d}	0.15^{cd}	0.21^{ab}	0.25^{a}	0.01	***
Intestinal Length (cm)	224b^{cd}	250^{a}	237^{ab}	235^{ab}	231^{bc}	234^{b}	214^{d}	213^{d}	217^{cd}	4.15	***

•abcdef Means with different superscript within the same row are significantly (P<0.001) different
NS: No significant difference (P>0.05). * = (P<0.05) ** = (P<0.01) *** = (P<0.001)

was observed with the liver as reported by Fasina *et al.* (2004) and Odunsi *et al.* (2006). Atteh (2004) also reported reduced liver weight with enzyme supplementation. The increased fat deposit observed in the control diet and other diets could be attributed to the higher bioavailable ME levels which could be converted to fats in the control and Maxigrain® supplemented PKM diets relative to the diets without Maxigrain® as reported also by Akpodiete *et al.* (2006).

Conclusion: When Maxigrain® was applied by supplementation, the maximum impact on broiler performance was observed to be between 10 and 20% diets which were better than the control diet in both starter and finisher phases. However, PKM at all levels (10, 20, 30, 40%) with or without Maxigrain® treatment did not result in any deleterious effect on overall broiler performance. The carcass analysis did not show any increase in the economic parts of the breast, thigh and drumstick with or without Maxigrain® irrespective of the method of application. The economic analysis reveal a downward reduction in cost of feed (N/kg) with increasing level of PKM inclusion. The health of the birds was not affected and mortality was not significant throughout the study.

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Evaluation of Enzyme (Maxigrain®) Treatment of Graded Levels of Palm Kernel Meal (PKM) on Nutrient Retention

A.A. Sekoni*, J.J. Omage, G.S. Bawa and P.M. Esuga
Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria

Abstract: A nutrient retention trial was conducted over a twenty four day period. Eighty one day old chicks of Arbor acres strain were randomly allotted to nine isonitrogeneous dietary treatments where PKM was included in the diet at 0,10,20,30 and 40% levels and PKM treated with Maxigrain® at 10, 20, 30, and 40% levels with three replicates and three birds each in metallic cages. Results show that there was significant ($P<0.001$) difference in protein, fat, NFE and metabolizable energy retention which were higher in the control and Maxigrain® treated diets compared with the corresponding diets without Maxigrain®. The crude fibre retention was significant ($P<0.05$) lower in the control compared treatments. The crude fibre retention values at 20 and 30% PKM diets with Maxigrain® were significantly ($P<0.05$) lower than values for 20 and 30% PKM diets without Maxigrain®. The results indicates that enzyme treatment of PKM increased the retention of vital nutrients and metabolizable energy.

Key words: Palm kernel meal, Maxigrain®, broiler, nutrient retention, metabolizable energy

Introduction

The role of enzymes as feed additive in poultry diets is well established. Hastings (1946) and Allen *et al.* (1997) all observed that enzyme addition to monogastric animal feed reduced viscosity of ingesta in the intestine and showed a marked improvement on the various morphological effects of feeding fibrous materials to non-ruminant animals. The use of PKM in poultry diets has been practiced for several decades. Its low level of key essential amino acids (Lysine and Methionione in particular), high dietary fibre (particularly in the form of β-mannan) and grittiness have precluded its inclusion in broiler diets. Nutritionally, Palm Kernel Meal contains moderate amounts of protein and carbohydrate. Crude protein of Palm Kernel Meal ranges from 14 to 21%. This level is too low for use in starter diets for young chicks but it is adequate for older birds. Table 1 Show the physical characteristics and nutrient composition of PKM, while Table 2 shows the amino acid availability, exceeding 85%, except for valine and glycine.

Examination of the physical characteristics of Palm Kernel Meal reveals that its bulk density is quite high compared to copra meal (coconut) their respective values are 0.67 and 0.56g/cc. Although Palm Kernel Meal and copra meal have some common properties in carbohydrate composition, their water holding capacity differs greatly. Palm Kernel Meal water holding capacity is half the water holding capacity of copra meal (Sundu *et al*., 2005). These two physical characteristics, bulk density and water holding capacity, are important as they affect feed intake (Kyriazakis and Emmans, 1995).

A detailed description of the carbohydrate fraction of PKM has been reported by Knudsen (1997). This author reported that total carbohydrate of Palm Kernel Meal, excluding lignin was 50%, of which only 2.4% was of low molecular weight and 1.1% was starch while 42% is in the form of non-starch polysaccharides (NSPs) (Knudsen, 1997). This means that 81% of Palm Kernel Meal Carbohydrate is in the form of NSPs. Of the total NSPs present in Palm Kernel Meal the main form is an insoluble non-cellulose polysaccharide, accounting for 33.6% of the dry matter. The main sugars in the soluble non-cellulose polysaccharide were mannose and galactose while the sugars in the insoluble non-cellulose polysaccharide were mannose and glucose (Knudsen, 1997). The high amount of lignin (13.6%) in PKM, due possibly to the contamination with nut shell (Knudsen, 1997), makes this feedstuff feel gritty and fibrous. Fractionation of Palm Kernel Meal based on its proximate analysis reveals that 49% of the dry matter of Palm Kernel Meal is in the form of nitrogen free extract (Sue, 2001; Sundu *et al*., 2004). Of the NSPs present, it has been found that 78% is linear Mannan with very low galactose substitution, 12% cellulose, 3% glucoronoxylans and 3% arabinoxylans (Dusterhoft *et al*., 1992). It also contains small amount of galactomannan (Daud and Jarvis, 1992; Dusterhoft *et al*., 1992; Knudsen, 1997). This type of Mannan is characterized as hard and water insoluable (Warren, 1996). Most Palm Kernel Meal mannan is extremely hard, highly crystalline and water insoluble (Aspinal, 1970). However, Dusterhoft *et al.* (1992) reported that about 66% of Palm Kernel Meal mannan could be solubilized by sequential extraction with alkali and sodium chloride.

The metabolizable energy of palm kernel meal varies widely, from at least 2591 cal/kg (Chin, 2002) to 3959 cal/Kg (Sundu *et al*, 2005). This may be due to the fact that the oil content of palm kernel meal varies due to

differences in oil extraction process. The higher metabolizable energy values may be due to higher oil content remaining in the Palm Kernel Meal after the product is processed by expeller machinery (O'Mara *et al.*, 1999). MAXIGRAIN® - contains cellulose-10,000 i.u, Beta glucanase-200 i.u, Xylanase-10,000 i.u. Phytase-2500 FTU. Cellulase breaks down cell-wall for more energy and relocked nutrients. Xylanase and β-glucanase degrades non-starch polysaccharides in feeds. Phytase efficiently releases bound phosphorus from plant phytates and also liberates minerals and amino acids.

Numerous researchers have demonstrated that the soluble-NSP fraction, not the total NSP fraction, is responsible for anti-nutritive responses. These NSPs can bind large amounts of water, and as a result, the viscosity of fluids in the digestive tract is increased. The increased viscosity causes problems in the small intestine because it reduces nutrient availability (Particularly fat) and results in increased amounts of sticky droppings. (Choct, 1998).

To counteract these anti-nutritional effects, enzymes are often added to the feed. This study was therefore designed to evaluate the nutrient retention profile of broilers fed graded levels of PKM treated with and without Maxigrain®.

Materials and Methods

Eighty one day old broiler chicks (mixed sexes) of Arbor acres strain was used for this experiment. The birds were weighed at day old and brooded and fed a controlled diet shown in Table 3, during a two weeks adaptation period.

At two weeks of age, they were randomly assigned to nine groups housed in metallic cages and fed nine isonitrogeneous experimental diets formulated at 23%CP. The treatments were replicated three times with three chicks per cage. The experimental diet was fed during a 7-day pre- faecal collection period. This was followed by a 3 day excreta collection period using the total collection procedure. The excreta collected was oven dried at a temperature of 70°C for 48 hours. Weighed and ground prior to chemical analysis.

Feed and water was supplied *ad-libitum* during the trial period which lasted 24 days.

The treatment diets for the experiment were:

Treatment 1 – control diet without PKM or Enzyme
Treatment 2 – contain 10% PKM with Enzyme treatment
Treatment 3 – contain 10% PKM without Enzyme
Treatment 4 – contain 20% PKM with Enzyme treatment
Treatment 5 – contain 20% PKM without Enzyme
Treatment 6 – contain 30% PKM with Enzyme treatment
Treatment 7 – contain 30% PKM without Enzyme
Treatment 8 – contain 40% PKM with Enzyme treatment
Treatment 9 – contain 40% PKM without Enzyme

The PKM was treated with 100gm per metric ton of Maxigrain® and added at 10%, 20%, 30%, 40% inclusion of PKM to the diets. The compositions of the diets are as shown on Table 3. Samples of basal diet, test diets and excreta were analyzed for moisture, ether extract and crude fibre, using the method of AOAC (1990).

Table 1: Physical characteristics and nutrient content of palm kernel meal

Fractions	Composition	References
Dry matter (%)	94	(1)
Crude protein (%)	14-21	(1) (2) (3)
Gross Energy (K.Cal/kg)	4998*	(1)
Crude fibre (%)	21-23	(1) (4)
Lipid (%) 17-Aug	(1) (4)	
Ash (%) 6-Mar	(1) (4)	
Bulk density (unmodified) (g/cm^3)	0.67	(1)
Bulk density (0.5mm) (g/cm^3)	0.57	(1)
WHC (1mm) (g water/g feed)	2.82	(1)
WHC (0.5mm) (g water/g feed)	2.93	(1)

*WHC: Water holding capacity. (1) Sundu *et al.*, 2005; (2) Nwokolo *et al.*, 1976; (3) Onwudike, 1986, (4) Sue, 2001.

All data obtained were subjected to the analysis of variance using the General Linear Model procedure of Statistical Analysis System (SAS) computer software package (1985). Significance of difference between means was determined by applying the Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

The Apparent Metabolizable Energy (AME), True Metabolizable Energy (TME) and Nitrogen Corrected Metabolizable Energy (MEn) of each diet were calculated using the following equations.

AME = Ge_f x Quantity of feed consumed – GE_{fa} x Quantity of faecal output.

TME = Ge_f x Quantity of feed consumed – GE_{fa} x Quantity of excreta of fed bird – GE_{fa} x Quantity of excreta of fasted birds

MEn = Ge_f x Quantity of feed consumed – GE_{fa} x Quantity of faecal output. ± (8.22 X Nitrogen retained).

Where Ge_f = Gross Energy of feed in Kcal/kg
Ge_{fa} = Gross Energy of faeces in Kcal/kg

Nutrient retention (NR) was determined for crude protein, fat, crude fibre, ash, and NFE, using the equation.

$$N.R = \frac{\text{Nutrient Intake} - \text{Nutrient output}}{\text{Nutrient Intake}} \times 100$$

Where: Nutrient Intake (g) = Dry feed intake x Nutrient in diet

Nutrient Output (g) = Dry faecal output x Nutrient in faeces.

Results

The effects of PKM with and without Maxigrain® treatment on nutrient retention is presented on Table 5. The protein retention in the control, 20, 30 and 40% Maxigrain® treated diets and 10 and 20% diets without Maxigrain® were similar but significantly ($P<0.001$) higher than values for 30, 40% diets without Maxigrain®

Table 2: Amino acid composition and availability of Palm Kernel Meal (percentages)

Amino Acids	Composition (%)			Availability	0-3 weeks of broiler
	(A)	(B)	(C.)	(%) (B)	Requirements (D)
Arginine*	2.18	2.68	2.40	93.2	1.25%
Cystine	0.20	-	-	-	(Cys + Meth) 0.90
Glycine	0.82	0.91	0.84	63.3	(Glycine + Serine) 1.25
Histidine*	0.29	0.41	0.34	90.1	0.35
Isoleucine*	0.62	0.60	0.61	86.1	0.80
Leucine*	1.11	1.23	1.14	88.5	1.20
Lysine*	0.59	0.69	0.61	90.0	1.10
Methionine*	0.30	0.47	0.34	91.0	(Cys + Meth) 0.90
Phenylanine*	0.73	0.82	0.74	90.5	(Phenyl + Tyrosine) 1.34
Threonine*	0.55	0.66	0.60	86.5	0.80
Tyrosine	0.38	0.58	0.47	85.0	(Phenyl + Tyrosine) 1.34
Serine	0.69	0.90	0.77	88.7	(Glycine + Serine) 1.25
Valine*	0.93	0.43	0.80	68.4	0.90
Tryptophan*	0.17	-	0.19	-	0.20

and 40% with Maxigrain®. Protein retention of the 10% Maxigrain® treated diet show best results compared with control and other treatments with or without Maxigrain® treatment. PKM supplies both protein (CP 18%) and gross energy (4998kcal/kg) which is classified as protein source of medium grade. The superiority of protein retention at all levels of PKM treated with Maxigrain® compared to the PKM without enzymes indicated that Maxigrain® improves the protein content of PKM.

Fat retention profile were significantly ($P<0.001$) different across the treatments. The values for the control, 10 and 20% PKM with Maxigrain® were significantly ($P<0.001$) higher than all other treatments. Fat retention in the Maxigrain® treated diets were similar in 10, 20 and 30% PKM levels followed by a decline in 40% PKM level. The fat retention values were similar in 40% PKM with Maxigrain® and 10, 20, 30 and 40% PKM without Maxigrain®.

The crude fibre retention in the control diet was significantly ($P<0.05$) lower than all other diets with or without Maxigrain®. The crude fibre retention increased with increasing levels of PKM with or without enzyme.

The ash retention was significantly ($P<0.001$) higher in 10% PKM with Maxigrain® than all other treatments. The values in the control, 20 and 30% PKM with Maxigrain® and 10 and 20% PKM without Maxigrain® are similar but significantly ($P<0.001$) higher than values for 40% PKM with Maxigrain® and 30 and 40% PKM without Maxigrain®.

The NFE retention in the control, 10, 20 and 30% PKM with Maxigrain® and 10% PKM without Maxigrain® were significantly ($P<0.001$) higher than all other treatments. Similarly, the NFE retention values for all levels of PKM with Maxigrain® were significantly ($P<0.001$) higher than the values for the corresponding levels of PKM without Maxigrain® except at 40% with and without Maxigrain® where the values similar.

The effects of PKM with and without Maxigrain® treatment on metabolizable energy is presented on Table 6. The values for the apparent metabolizable energy and nitrogen corrected metabolizable energy in the control and 10% PKM with Maxigrain® were significantly ($P<0.001$) higher than all other treatments. Similarly, the apparent metabolizable energy values for all levels of PKM with Maxigrain® were significantly ($P<0.001$) higher than the values for corresponding levels of PKM without Maxigrain® except at 40% with or without Maxigrain® where the values were similar. The true metabolizable energy (True ME) values in the control and all levels of PKM with Maxigrain® are significantly ($P<0.001$) higher than all levels of PKM without Maxigrain® except at 40% with and without Maxigrain® where the values were similar.

Discussion

The effects of Maxigrain® treatment of PKM on nutrient retention showed that protein retention between diets with Maxigrain® treatment were higher compared to diets without Maxigrain® which could have contributed to the higher body weight gains observed among birds fed Maxigrain® treated diets maximized with optimum at 20% PKM inclusion compared with control and other diets with or without Maxigrain®. This agrees with the findings of Ariff Omer *et al.* (1998) who reported optimum PKM inclusion rate in poultry ration at 20%.

There was significant variation in fat retention among all treatment, which is higher in the control and Maxigrain® treated diets compared with diets without Maxigrain®. This is in agreement with Marquardt *et al.* (1996) who reported improvement in the body weight and feed conversion efficiency due to an increase in fat and protein digestibility. Increase fat retention also increases the bioavailability of fat soluble vitamins. This was easily observable in the increase in ash retention in Maxigrain® treated diets compared with diets without Maxigrain®. The observation of Swain and Johri (1999) that the addition of enzyme to feed caused increase in

Table 3: Percentage composition of starter diets

Ingredient	Control	10%	20%	30%	40%
Maize	55.00	47.50	40.00	33.00	25.00
Soyabean Meal	40.00	37.50	35.00	32.00	30.00
*PKM	0.00	10.00	20.00	30.00	40.00
Bone meal	2.60	2.60	2.60	2.50	2.60
Limestone	1.60	1.60	1.60	1.60	1.60
Salt	0.35	0.35	0.35	0.35	0.35
**Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Cost/kg diet(N)	43.00	40.00	38.00	35.00	33.00
Calculated analysis of starter diets					
ME Kcal/kg	2800.00	2757.00	2657.00	2561.00	2456.00
Crude Protein(%)	23.00	23.00	23.00	23.00	23.00
Crude Fibre(%)	4.00	5.00	5.40	6.00	7.00
Ether Extract(%)	3.50	3.80	4.00	4.20	4.50
Lysine (%)	1.35	1.33	1.30	1.30	1.30
Methionine(%)	0.44	0.45	0.46	0.48	0.50
Calcium (%)	1.50	1.50	1.50	1.50	1.50
Available Phosphorus(%)	0.70	0.70	0.70	0.70	0.70

**Bio-mix premix supplied per kg of diet: Vitamin A 12500 I.U Vit D_3 2500 I.U Vit E 50mgL Vit K_3 2.5mg; Vit B_1, 3.0mg: Vit B_2 6.0mg; Vit B_6 6.0mg: Niacin 40.0mg; calcium pantothenate 10.0mg; Biotin 0.80mg; Vit B_{12} 0.25mg; Folic acid 1.0mg; Chloline chloride 300mg; manganese 100mg: Iron 50mg; Zinc 45; copper 2.0mg; cobalt 0.25mg; iodine 1.55, selenium 0.1mg.

Table 4: Percentage composition of finisher diets

Ingredient	Control	10%	20%	30%	40%
Maize	65.00	55.00	50.00	40.00	32.00
Soyabean Meal	30.00	30.00	25.00	25.00	23.00
*PKM	-	10.00	20.00	30.00	40.00
Bone meal	2.60	2.60	2.6.00	2.60	2.60
Limestone	1.60	1.60	1.60	1.60	1.60
Salt	0.35	0.35	0.35	0.35	0.35
**Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Cost/kg diet(N)	40.00	38.50	35.00	33.00	31.00
Calculated analysis					
ME Kcal/kg	3000.00	2850.00	2758.00	2635.00	2528.00
Crude Protein (%)	20.00	20.00	20.00	20.00	20.00
Crude Fibre (%)	3.00	4.00	5.00	6.00	7.00
Ether Extract (%)	3.70	3.90	4.10	4.30	4.50
Lysine (%)	1.10	1.14	1.00	1.00	1.00
Methionine (%)	0.39	0.42	0.42	0.40	0.45
Calcium (%)	1.50	1.50	1.50	1.50	1.50
available Phosphorus (%)	0.60	0.60	0.60	0.60	0.60

**Bio-mix premix supplied per kg of diet: Vitamin A 12500 I.U Vit D_3 2500 I.U Vit E 50mgL Vit K_3 2.5mg; Vit B_1, 3.0mg: Vit B_2 6.0mg; Vit B_6 6.0mg: Niacin 40.0mg; calcium pantothenate 10.0mg; Biotin 0.80mg; Vit B_{12} 0.25mg; Folic acid 1.0mg; Chloline chloride 300mg; manganese 100mg: Iron 50mg; Zinc 45; copper 2.0mg; cobalt 0.25mg; iodine 1.55, selenium 0.1mg.
*The PKM based diets consists of 10%, 20%, 30% and 40% with Maxigrain® and 10%, 20%, 30% and 40% without Maxigrain®.
One Naira (N) is equivalent to 128 US Dollars ($)

energy but a reduction in fat retention is not in agreement with this study since retention of fat relates to vitamin storage in the body, a reduction may be detrimental to broilers. The results of experiments conducted by Langhout and Schutte (1995) showed that the effects of chick performance and nutrient digestibility of dietary endo-xylanase in wheat and rye based diets are influenced by the type of fat in the diet. The crude fibre retention was poorest in the control as there was no enzyme or PKM in the diet. Retention of crude fibre was lower in 20 and 30% Maxigrain® treated diets compared to the correspondingly diets without Maxigrain®. Even at 10 and 40% PKM levels with and without Maxigrain® where crude fibre retention values were not significantly different, the values for the Maxigrain® treated diets were numerically lower. This

Table 5: Effects of treated PKM with and without Maxigrain® on nutrient retention of broiler chicks

Parameters	Control	-------- PKM with Maxigrain® Diets --------- 10+	20+	30+	40+	-------- PKM without Maxigrain® Diets -------- 10-	20-	30-	40-	SEM	Signifi-cance
Protein	54.333^{bc}	64.667^{a}	58.393^{ab}	51.653^{bcd}	47.28^{cde}	50.94^{bcd}	47.00^{cde}	44.643^{de}	42.653^{e}	1.41	***
Fat	79.937^{a}	78.333^{ab}	71.91^{bc}	70.797^{cd}	62.387^{ef}	59.637^{f}	63.927^{def}	66.33^{cdef}	68.667^{cde}	1.34	***
Crude Fibre	17.163^{a}	25.997^{b}	28.333^{b}	48.00^{c}	54.417^{d}	28.00^{b}	48.667^{c}	54.323^{d}	56.333^{d}	0.90	*
Ash	33.00^{b}	42.00^{a}	35.333^{b}	31.67^{b}	26.00^{c}	34.33^{b}	33.00^{b}	26.00^{c}	21.67^{d}	0.78	***
NFE	71.00^{ab}	75.667^{a}	74.667^{a}	66.00^{bc}	52.00^{ef}	68.667^{b}	61.00^{cd}	55.333^{de}	49.00^{f}	1.10	***

· Abcdef Means with different superscript within the same row are significantly (P<0.001) different. · *= (P<0.05) *** = (P<0.001)

Table 6: Effects of treated PKM with and without Maxigrain® on metabolizable energy of broiler chicks

Parameters	Control	-------- PKM with Maxigrain® Diets --------- 10+	20+	30+	40+	-------- PKM without Maxigrain® Diets -------- 10-	20-	30-	40-	SEM	Signifi-cance
Apparent ME	3496.7^{a}	3407.00^{a}	3017.00^{b}	2724.70^{bc}	1742.00^{de}	2839.00^{b}	2439.33^{c}	2035.0^{d}	1641.70^{e}	71.63	***
Men	3498.7^{a}	3409.0^{a}	3019.0^{b}	2725.7^{bc}	1744.0^{de}	2841.0^{b}	2441.3^{c}	2037.0^{d}	1643.7^{e}	71.63	***
True ME	3402.7^{a}	3315.3^{ab}	2922.0^{bc}	2625.3^{cd}	1640.3^{f}	2593.7^{cd}	2276.0^{de}	1867.3^{ef}	1525.0^{f}	79.83	***

•abcdef Means with different superscript within the same row are significantly (P<0.001) different. • *** = (P<0.001), Apparent ME = Apparent Metabolizable Energy. • Men = Nitrogen Corrected Metabolizable Energy. • True ME = True Metabolizable Energy

indicates that Maxigrain® a cocktail of enzymes must have broken down some of the NSPs in the PKM. Of the NSPs in PKM, 78% is linear mannan, 12% cellulose, 3% glucoronoxylans and 3% arabinoxylans (Dusterhoft *et al.*, 1992). Since Maxigrain® contains cellulose, β-glucanase, xylanase and phytase, these enzymes must have acted on cellulose, glucoronoxylans and arabinoxylans thereby reducing the crude fibre content and subsequently increasing the energy content retention and NFE. The NFE represent the soluble carbohydrates in the diet. The addition of enzymes to PKM is expected to ameliorate the antinutritive effect of β-mannan and also to degrade large percentage of NSPs and oligo-saccharide components of the diet. (Akpodiete *et al.*, 2006) The higher values of NFE retention in PKM diets with Maxigrain® as compared with PKM diets without Maxigrain® can be attributed to the degradative effect of Maxigrain® on the NSPs of PKM with subsequent release of soluble carbohydrates.

The effect of Maxigrain® treatment on ME of broiler chicks show increase in bioavailable energy (AME, TME and MEn) in the PKM diets treated with Maxigrain® to a maximum inclusion of 30% PKM. Atteh (2000) had earlier observed improvement in crude fibre digestion and ME of diets where enzyme supplementation of 50% wheat bran replaced 50% of maize in a control diet relative to an unsupplemented wheat bran diet. The AME and MEn of the different diets and the control with or without Maxigrain® show little variation. This support the work of Sibbald and Slinger (1962) who observed a close correlation between corrected and classical ME values and therefore stated that since the amount of tissue protein which is catabolized is small relative to the amount stored by growing birds, or deposited in eggs of laying hens, the imposition of penalty of nitrogen retention is a questionable procedure. Swift and French (1954) argued that since storage of protein characterize growth, it is difficult to justify the imposition of penalty for nitrogen retention. Muztar *et al.* (1977) concluded that application of nitrogen correction does not seem to be of major consequence when one considers the amount of time and labor in determining the nitrogen content of feed and excreta samples. Therefore the little variations observed between the AME and MEn of the different diets in this study support the earlier works that believe that the exertion of penalty of nitrogen correction of energy was not necessary.

Conclusion: The improvement in protein, fat and NFE retention was observed to be more significant than crude fibre retention. The Apparent metabolizable energy (AME), True Metabolizable energy (TME), and Nitrogen corrected metabolizable energy (MEn) of the PKM treated diets show improvement higher than diets without PKM treatment. Maxigrain (a cocktail of exogenous enzymes) treatment improves nutrient retention on a relative bases but to maximize the benefit of enzyme supplementation of PKM, a more specific cocktail of enzymes containing mannanase must be included.

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