Discoveries in Agriculture and Food Sciences - Vol. 13, No. 03
Publication Date: May 25, 2025
DOI:10.14738/tnc.1303.18761.
Flores, D. A. (2025). Nutraceuticals for Functional Feeding and for Drug Discovery in Ruminant Production: A Commentary Paper. Discoveries in Agriculture and Food Sciences, 13(03). 14-22.

Nutraceuticals for Functional Feeding and for Drug Discovery in Ruminant Production: A Commentary Paper

D. A. Flores
Skye Blue (SB) Internet, 1440 Barberry Drive
Port Coquitlam, BC, Canada V3B 1G3

ABSTRACT

The paper lists nutraceuticals from the literature and the world-wide web according to their definition and benefits to nutrition and health. The following nutraceuticals are both novel or have received attention in the literature. They are anti-oxidants, water-soluble carbohydrates (WSC), soluble dietary fibre (SDF), biogenic peptides, functional amino acids (FAA), probiotics, vitamins as pharma, minerals, phytochemicals, "greens" such as herbs, weeds and green forages, omega-3 fatty acids, dietary "bulk" fibre, phytonutraceuticals and adaptogens or anti-stress compounds. The paper then discusses selected topics encountered in the literature or that is still being proposed for the lab bench. They are the: sugars of WSC and SDF, peptides from feed proteins as prebiotics for the rumen stomach and hindgut, prebiotic "bulk" fibre, VitD2,3 analogues as agonists and milk food proteins (MFP). Drug development is suggested of the type by mining the transcriptome and its transcription factors (TF) and the use of peptide nucleic acid (PNA) - based fine-biochemical agents that can be directly applied for therapeutic purposes versus another approach using the low molecular weight (LMW) – proteome in plasma, tissues and secretalogues to find biopharma. An actual e. g. described here not necessarily derived from nutraceuticals but illustrating the use for PNA-carriered TF is to enhance vaccines against Streptococcus pneumoniae, that is, using MR1 protein molecules that activate mucosa-associated invariant T (MAIT) cells in a humeral response against the bacteria that causes pneumonia to be direct applied (DA) by nasal spray applicator. With new perspectives from nutraceuticals, it should be possible to derive new pharma through research drug pipelines to cure, manage incidence or prevent diseases.

Keywords: Nutraceuticals, Feeding, Pharma, Transcriptomics, Ruminants, Animal Production.

INTRODUCTION

According to A. Pandey et al. (2023) benefits in animal efficiency, animal health and sustainability in production systems can result with nutraceutical feeding such as probiotics and prebiotics, including enzymes and herbs or spices (extracts or additives) of which the latter can stimulate feed intake, endogenous secretions or have anti-microbial activity; they also mentioned that herbal additives are an effective substitute due to the ban on antibiotic with antibiotic resistance and residuals present in animal products. The additional medicinal properties of nutraceuticals are many; they include anti-microbial, anti-inflammatory, anti-oxidant, digestibility and immune-stimulating activity; there are also other issues to address in sustainable feeding practices in agriculture (A. Pandey et al., 2023).

The paper presents a recent listing of nutraceuticals to augment available listings from the published literature and world-wide web (WWW) for both their definition and to describe their use for both nutrition and health.

MAJOR NUTRACEUTICALS DEFINED BY CLASSIFICATION

The listing together with the scientific literature represents nutraceuticals that are still novel or have already received attention are defined and further described below:

1. **Anti-oxidants** are defined as organo-chemicals, e. g. s. of which are: polyphenols, flavonoids, catechins and isoflavones from both plant and animal sources whose mechanisms of action are as scavengers of free radicals and also chelate metal ions.
2. **Prebiotics as Water-Soluble Carbohydrate (WSC) and Soluble Dietary Fibre (SDF)**: Defined as compounds that confer indirectly or directly a nutritional or health benefit to the host animal, an e. g. of which are the oligosaccharides of plant origin, which confer nutritional benefit in the rumen stomach of livestock including improved fibre digestion, protein microbial cell synthesis and short-chain fatty acid (SCFA) production.
3. **Prebiotics as Peptides:** Define here as shorter amino acid sequences also called oligomers that play a role proteinergically for microbial cell protein (MCP) synthesis and biogenic role in health maintenance and treatment. Most peptides currently dealt within clinical nutrition are as nutraceuticals relating to cardiac health from plant vegetables like beets and seafood meats like fish as with tuna and peptides derivable from milk food proteins (MFP).
4. **Functional Amino Acids (FAA**): These are defined outside the amino acid building blocks that make up proteins but have auxiliary functions endocrinologically for production and reproduction, immunologically for health and intake control and energy regulation.
5. **Probiotics**: Defined as live microorganisms that confer a nutritional benefit in the rumen by improving digestibility, acid-base balance, and controlling bloat, as examples. Also, they confer health benefits on the host in that they can restore, by stimulation, a balance in the growth of good bacteria while reducing the effect of harmful bacteria of the lower gut.
6. **Vitamins as Pharma**: As defined here, rather than focus on each of the Vitamins as such we refer here to their derivative compounded complexes, experimental or theoretical, that have been introduced, potentially with significant nutritional or health benefits, e. g. of which are: Protein Nucleic Acid (PNA)-Vit B12 carriered fine-biochemical agents, agonists in the activation of Vit D2,3 and theoretically PNA-Vit K2 (proposed here as a pentaquinone). There are new emerging anti-microbials that are nondependent as antibiotics are due to antibiotic resistance.
7. **Minerals**: Defined here as those specific minerals of which are mentioned here: Ca^{2+}, Sr^{2+}, and Zn^{2+} with applications in osteoporosis prevention and treatment and in the cows' condition referred to as "milk fever" during the transitional phase at the beginning of lactation. These are contrasted to other heavy metal minerals which are anti-nutritional in significance and are removable with chelating agents in the blood.
8. **Phytochemicals** – e. g. s. of these nutraceuticals are the: carotenoids, indoles, glucosinolates, organosulfur compounds, phytosterols, polyphenols and saponins belonging to various families of compounds and can have potential action across

different prevention and therapeutic health conditions. Strictly speaking, they are non-nutritive and are classified between food and pharma for maintenance or promoting health.

9. **“Greens” as Herbs and Weeds and Green Forage**: These are defined as green weeds that can be found, for e. g., in the roadside or on rice paddy bunds provided to teathered livestock with access containing bioactive compounds surmised to hypothetically provide anti-bacterial properties and/or stimulate immunity in the lower gut to control pathogenic bacteria and prevent incidence of disease. Additionally, amongst other forage, they also provide so-called “long fibres” which are believed to aid digestion of fibre and intake as a “bulk” nutritional which are lacking in low-quality feeds or byproduct concentrate feeding.
10. **Omega-3 Fatty Acids**: These are defined as dietary fatty acids of particular unsaturation in a structure of 18-22 carbon lengths with multiple double bonds at the 1st and the 3rd and 4th carbon atoms. There are 3 types: alpha-linolenic acid (ALA) from plants, eicosapentaenoic acid (EPA) from seafood and docasahexanoic acid (DHA) from seafood, algae, animal (meat, dairy and eggs), and plants (seaweed, nuts and vegetables) which help in preventing chronic disease conditions including atherosclerotic arterial conditions and aid in cardiovascular function.
11. **Dietary 'Bulk' Fibre –** defined as digestible fibre in livestock diets that promote SCFA production in the lower gut, e. g. butyrate, and when fed as the derivative butyramide, has been observed to have health-conferring effects from improved immunity and contributing to the animal’s welfare.
12. **Phytonutraceuticals**: Spices are referred to here as to their health-conferring effects as nutri-pharma to prevent, treat and manage diseases such as strengthening the immune system, for nutrition, control of blood sugar and cholesterol levels, anti-inflammatory or chronic conditions, as anti-oxidants, anti-obesity, mental conditions and cancer. E. g. s. of spices or condiments are ginger, cayenne pepper, cinnamon, black pepper, turmeric, fenugreek, rosemary and garlic. E. g. s. of bioactive compounds themselves can be specified but are not delved further into here.
13. **Adaptogens or Anti-stress Compounds**: A recent study featured so-called herbal-based adaptogens still of unknown modality or mechanism in poultry birds; because of heat stress applied cyclically with controls, feed intake was stimulated previously depressed, registered better body weight and better feed conversion ratio (FCR) with use of adaptogen supplementation in the diet of birds (S. Kumar et al., 2015)

A DISCUSSION OF SELECTED NUTRACEUTICALS

This section deals with a selection of topics on nutraceuticals that this author features here for the science literature or has already been considered for the lab bench.

Sugars of Water-soluble Carbohydrates (WSC) and Soluble Dietary Fibre (SDF) as Prebiotics for the Rumen Stomach and Hindgut

Introduced here are the ff. points: the issue of marker-assisted selection (MAS) for breeding of crop plants for feed to produce high sugar grasses (HSG), high in water-soluble sugars (WSC), the fact that there are different effects of WSC (and peptides) in the upper (e. g. rumen stomach in livestock models) and lower (lower bowel of human models) tract and that oligosaccharides can produce benefits in high-producing cows in early lactation from the diet.

Analysis of the WSC fraction from feed nutraceuticals as it affects digestibility and fermentation shows the following. WSC and the other fraction of soluble dietary fibre (SDF) made up of monosaccharides (e. g. glucose, fructose) and disaccharides (e. g. sucrose), fructan oligosaccharides (FOS) (e. g. inulin), galactan oligosaccharides (GOS), and cell wall (CW) saccharides like pectin, beta-glucan, and pentosans or gums (which can be analyzed for from the neutral detergent soluble fibre) have a positive effect on fibre digestibility found in lower gut studies in human models and less studied in the upper tract of rumen stomachs of livestock (Z.-W. Guan et al., 2021). Also, optimum levels in dairy may require inclusions of up to 25% in WSC in high- producing lactating dairy cows (A. Kasperowicz et al., 2014).

Although quite lengthy, this discussion continues dealing with so-called HSG containing metabolically upregulated levels of WSC revealing the need to employ new or ongoing crop improvement with grass and legume forages for grazing animals including high producing dairy cows. Of the options available for existing non-GMO avenues there are: 1) cross-breeding ryegrasses varieties that are HSG, e. g. of which are a) Sweet Spot ® and b) Aber ®, 2) protoplasmic fusion, 3) marker-assisted selection (MAS) for crop improvement and 4) initiating new programmes using either of the preceding methods also for tropical HSG varieties.

Breeding programmes will aim to address two major factors of: 1) biotic/abiotic stressors for better survivability in extreme weather, drought tolerance, cold tolerance, competitively crowding by weeds and with no extra fertilization required; and 2) agronomic practices for high biomass yield, higher regrowth yield, yield from bigger plots, intermixed sowing possible in a mix with 55% HSG content, wide soil pH range (5.5-8.5), limiting excretion by ruminants in 24% less N in urinary and faecal output, improvement in digestibility and protein conversion, increased dry matter intake (DMI) and increased energy/protein nutrition for better animal management and welfare or well-being.

Peptides from Feed Protein as Prebiotics for the Rumen Stomach and Hindgut

As of yet, nobody knows the exact functionality peptide prebiotics when successfully translocated to the hindgut and post-absorptively. Perhaps endproducts that are biogenic can be fed as stable derivatives, reaching there.

An example of biogenic peptides from milk-derived proteins have recently been identified to be antihypertensive, anti-diabetic, cytomodulatory, antioxidative, antimicrobial, and mineral binding (P. Kashung and D. Karuthapandian, 2025).

The biogenic peptides not dealt with into in this paper, are as drug target candidates as searched for in the low molecular weight (LMW)-proteome of plasma rather than the targeted approach of RNA gene-silencing with PNA-carriered technology as fine-biochemical agents. Furthermore, we will not delve into the considerable discussion on peptides of rumen microbial digestion and the reader is referred to it elsewhere (D. A. Flores. 2024a; D. A. Flores. 2024b).

Prebiotic "Bulk" Fibre

This is a new concept for nutrition where "bulk" fibre has a specific health-conferring benefit to the animal in the hind gut due to the production of SCFAs, e. g. of which butyramide, a derivative nutraceutical which converts to butyric acid there and has a protective function against pathogenic bacteria directly or indirectly through immune function and can act against colon cancer.

At the molecular level, the organic acid butyrate also produced endogenously in colonocytes and works molecularly as a histone (basic proteins with chromosomal DNA) deacetylase (HDAC) inhibitor or signaling through G-protein-coupled receptors (GPCRs). The organic acid is also assumed to be absorbed as butyrate; butyrate is now receiving particular attention for beneficial effects on intestinal energy metabolism and the gut's lining's role with immunity (H. Liu et al., 2018)

Vit D2,3 Analogues as Agonists

Now presented here in this paper is a lengthy discussion on an approach for research by the author earlier begun in 1979 at Simon Fraser University, British Columbia Canada (see: D. A. Flores, 1980) with the aim at eventually developing an active VitD2,3 analogue as agonist intended for balancing immunity, preventing or treating osteoporosis and in lactating dairy cows treating 'milk fever' during their transition.

More research is proposed from earlier conversion synthetically of Lanosterol to its Lanosteral derivative and further derivatizing to the active form of VitD2,3 through the liver's and kidney's enzymes that reversibly, competitively feedback inhibit the natural metabolite or substrate and could be candidates as pro-VitD therapeutic drugs.

This research which started with Lanosterol to its Lanosteral derivative and is converted to the Cholesterol molecule via modified enzymes in their mutant hosts by the cytochrome P-450 superfamily of enzymes, amongst others (preceded by Flores' 7-step synthesis up to Lanosteral), then to 7-dehydrocholesteral, which upon irradiation coverts to cholecalciferal or the active analogue to VitD3 and to related hormones, calcifediol and calcidiol derivatives through the liver and kidney, respectively.

Enzymes upon mutational modification, tested for affinity and avidity to drug analogues, is open to experimentation with structure-function mechanisms to be studied with the enzymes involved in proteomics, structure-folding, X-ray crystallography and structure-proofing to make the Lanosteral side-chain derivatives bind constructively by amino acid residue replacement. Orientation and proximity, viz., hydrogen-bonding between the aldehyde group of the prosthetic side-chain and amino acid residues of the enzymes apply here.

The estimates of recovering fortuitous binding to the specifications of the aldehydic (-CHO) group to replace for the methyl (-CH3) group based on hydrogen-bonding (and van der Waals forces) in a less hydrophobic environment by however number of amino acids (or number of residues) and at what position(s), i. e. the probabilities required for this transformation to occur depend on the following criterion: 1) intramolecular bond length, 2) its angle(s), and 3) type according to strength of bonding in the enzyme(s) accommodating the variant aldehydic group (-CHO) of the model compound(s) synthesized.

Fungal and human microsomal hepatocyte and mitochondrial nephrocyte cells can be the source of mutant enzymes converting Lanosteral all the way to 7-dehydrocholesteral followed by a step via irradiation with UV-B thermal isomerization under the skin and then to calcidiol derivative, the first hydroxylation product from hepatocytes in liver followed by the second hydroxylation by enzymes from nephrocytes in kidney or hepatocytes in liver.

To derive the series of biosynthetic mutant enzyme "isoforms", a high-speed, throughput protocol is used with exposure to X-ray irradiation, the aliquot plated onto individual unicellular-derived colonies, each a homogenous colony, is then analyzed by semi-automated protocols (viz. sampling techniques, plating replication techniques) on clearing a lawn of substrate of sterol intermediates after starting and then to end product using mammalian cell culturing for each enzyme candidate. To test sequential reactivity each enzyme candidate individually and cumulatively is reacted in the sequence with the substrate on an enzyme impregnated filter paper disc on substrate-containing media plates and successful endproduct collected and detected by NMR and mass spectrophoto-metry (MS). The mutant or new enzyme candidates will be further characterized and screened by proteomic techniques.

It is presume that the series of end reactions to attain the active forms of VitD agonist are possible via UV-irradiation and further sequential hydroxylations by liver and kidney requiring flash-freezing due supposedly to the spontaneous free-radical breakdown or other oxidative reactions to effect their stabilization of the active forms readily available for administration clinically to either: 1) support acute care and/or 2) ongoing chronic cases in out clinics under both a doctor's supervision and nurse's administration. Steroidals such as those derived and proposed from VitD active forms can open up further speculation as to their applicability in areas of lower-intensity, systemic chronic inflammation (SCI) in heart health, type-2 diabetes milletus (T2DM), muscular conditioning and certain cancers (e. g. s. colon and breast).

VitD status has been implicated in the aetiology of T2DM, as a test case clinically. This is mediated in part by immune-modulating properties of the active forms, 1-alpha-25-dihydroxy VitD3 and 1, 25(OH)2 VitD3, which both can downregulate pro-inflammatory cytokines, particularly tumour necrosis factor alpha (TNF-alpha) and interleukin (IL-6). Additionally, these aspects with VitD status have been associated with both body mass index (BMI) and non-BMI related status. This is evidence other than calcaemic effects traditionally attributable to the vitamin that can be associated with health outcome.

To briefly outline the toxicological elimination of fat-soluble steroidal-like derivatives such as the VitD agonist described here where for: solubilization and then transport out of the cell is carried out to eliminate the toxic end products, the following steps are: 1) CYP protein process 1-20 molecules per sec. but are also inducible in the liver and can form a 'sizable' proportion of live protein (there are also toxins and bodily hormones to process); 2) further conjugation of products with Nrf2 translocation to the nucleus in response to CYP metabolism turns on with steroids and fat-soluble vitamins, comprising 4% of protein in liver, and also present amongst others in the kidneys; 3) products of GST conjugation can be excreted from the liver into bile and for elimination from the intestines; and they can also be excreted from kidneys where they are processed further and eliminated in the urine. Finally, ABC (ATP-binding cassettes) are transporters that act as toxins pumps through cell membranes, called MRPs (multi-drug resistance proteins).

Milk Food Protein (MFP)

There are mentioned in the literature 3 e. g. s. of MFPs that can be boosted due to their medicinal conferring properties: casein, alpha-lactalbumin and lactoferrin which could address conditions of: diabetes, metabolic syndrome, obesity and cardiovascular disease (CVD). The MFPs likely have scope for drug discovery around chronic inflammatory conditions related to various disease conditions. To take an e. g. by molecular mechanism, lactoferrin can migrate intracellularly to the nucleoli as evidenced by DNA immuno-staining or by immune-fluorescence. After entering through the nuclear membrane, presumably, and with exposure to lipopolysaccharide (LPS) from pathogenic bacteria, for e. g. E. coli, leads to activation of the nuclear TF NF-kappaB which binds DNA at the promoter, e. g. TNF-alpha, and with lactoferrin, leads to down-regulation of transcription of cytokine pro-inflammatory biomarkers decreasing binding. This has been modeled with studies on human monocyte blood cell lines (L. Haversen et al., 2003).

There is a suggestion made for small molecular drug discovery, that is, small ortho-/biomolecular drugs that can facilitate the binding to NF-kappaB TF at its operator and its DNA then leading to a 'suite' of down-regulated proinflammatory family of gene biomarkers or products such as: TNF-alpha, IL-factors, chemokines, MMP-9, VEGF, COX-2 and 5-LOX.

Apart from the MFP, there are other cardioprotective candidate functional foods. These are from: vegetables and fruits, nuts and seeds, seafoods such as fish, coffees, teas and dark chocolate, and whole grains with the intact kernels.

The MFP can be targeted for their health-conferring benefits for their effects on risk factors and protective mechanisms against them using small ortho- or biomolecular tools, e. g. biomarkers, or TF and their variants as possible candidates for drug discovery.

NEW DRUG DISCOVERY WITH PNA-CARRIERED TFS THROUGH TRANSCRIPTOMICS

As was referred to in the introduction, this paper serves as an update describing the significance of nutraceuticals and drug discovery studying the regulation of metabolism relating nutraceuticals and their mode of action to mining the transcriptome and DNA-based TFs for use eventually in new pharma as defined in these PNA-carriered fine-biochemical agents that can be administered for therapeutic uses (cf. directly applied on contact, DA; intramuscularly, IM; intravenously, IV; and intraperitoneally, IP). This is in contrast to use of techniques to mine the (LMW)-proteome, with peptides in plasma, in tissue or from secretalogues to find new drug biopharmaceuticals already as previously mentioned.

Transcriptomics like genomics is the field of RNA sequencing utilizing techniques for: 1) microarrays using only some or predetermined sequences and RNA-Seq or RNA sequencing using high-throughput sequencing of all sequences, to "back" analyze the DNA structure, viz., mapping the DNA genome; 2) how to and quantifying expression of mRNA levels and 3) measuring "biomarkers" called single nuclear polymorphisms (SNP) of genes in DNA of interest to specify their chromosomal locations and their relative distances. The significance of this field is to gather useful information from RNA "superposed" to the DNA backbone which is analyzed. DNA analyses results in labeling or annotation of structure and genetic function and describing gene regulation which takes perspectives for describing gene expression in different tissues, under different physiological conditions and their time course. The goal of utilizing transcriptomics here for new drug discovery has been to sequence mRNA transcripts as to coding for transcription factors (TF) for DNA regulation of gene expression for use in the synthesis of PNA-carriers in future.

An e. g. described here although not relating to nutraceuticals but to biopharmaceuticals and illustrating use of this type of PNA-carriered TF technology would be to enhance vaccines against Streptococcus pneumoniae tested with a system of microbial targets or pathogens, their production of antigens (Ag), a riboflavin metabolite for opsonization enhancement with IgG by MR1 molecules, that is, MHC class Ib-related protein 1 molecules, that activates mucosa-associated invariant T (MAIT) cells, for e. g. in the nasopharyngeal openings in cows and the liver, as would be the case with the application of vaccines and induced humoral adaptive antibacterial immunity (see: C. Boulouis et al., 2022) to treat those lung-associated diseases as pneumonia in cows or beef lot cattle to be DA via nasal spray applicator.

CONCLUDING REMARKS

This review presents possibly new perspectives through this recent collection of nutraceuticals presented here that could further stimulate advances for biomedical research through biomedical drug pipeline development of drug candidates for therapeutics that could in future spell possibly breakthroughs towards managing diseases.

DECLARATION OF CONFLICT OF INTEREST

The author declares no conflict of interest with this paper.

ACKNOWLEDGMENTS

The author wishes to thank with gratitude the provision of management, staff and personnel, the premises for Barberry House, Port Coquitlam, British Columbia Canada without which the work of this paper would not have been made possible.

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