Natural plant extracts: Classical remedies bring modern animal production solutions

Kamel C.

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Natural plant extracts: Classical remedies bring modern animal production solutions

C. Kamel
PANCOSMA, S.A., Voie-des-Traz 6, 1218 Le Grand-Saconnex, Geneva, Switzerland

SUMMARY – The new millenium has ushered in a re-evaluation of the way we feed and manage livestock. The end of the 20th century saw great advances in several areas in the animal production industry (i.e., genetics and environmental impact), but had serious consequences on consumer confidence after the BSE crisis in England, the dioxin scare in Belgium, and most recently, the Listeria outbreak in France. Food safety, as it relates to the maintenance of both animal and human health and welfare, now depends to a large extent upon the inclusion of feed components deemed "consumer-safe". In Europe, the growing unwillingness to rely upon standard solutions involving antibiotic or antimicrobial agents has given rise to several alternative strategies, such as that based on natural plant extracts and their intrinsic active principles. Due in part to today's technological advances, new state-of-the-art scientific methods are being used to re-examine these age-old substances, especially as it relates to their potential benefits and limits as valid alternatives for the future. Like their antibiotic predecessors, in the final analysis modern strategies will most likely not be based on one single answer, but on a combined solution of different types of products with well-described actions whose synergism yields benefits without threat of toxicity, end-product residues and microbial resistance.

Key words: Natural extracts, animal production, mode of action, benefits, risk analysis.

RESUME – "Extraits naturels de plantes : Remèdes classiques apportant des solutions modernes en production animale". Le nouveau millénaire commence par une réévaluation sur la façon dont nous nourrissons et élevons le bétail. La fin du 20ème siècle a vu de grands bouleversements au sein de quelques secteurs de l'industrie des productions animales (au niveau génétique et environnemental par exemple), mais cela a eu des conséquences graves sur la confiance du consommateur comme le montre la crise de la "vache folle" en Angleterre, le scandale de la dioxine en Belgique et plus récemment l'épidémie de Listéria en France. La sécurité alimentaire est liée au maintien de la santé et du bien-être aussi bien de l'homme que de l'animal. Ceci dépend en grande partie de l'utilisation d'additifs dans l'alimentation animale totalement sûrs et sains pour le consommateur. En Europe, le refus grandissant d'utiliser des solutions standards telles que les antibiotiques et les agents anti-bactériens a favorisé le développement de quelques stratégies alternatives, en particulier celles basées sur les extraits naturels de plantes et leurs principes actifs intrinsèques. Grâce, entre autre, aux dernières avancées technologiques, de nouvelles méthodes scientifiques ont été utilisées pour ré-examiner ces vieilles substances en particulier en ce qui concerne leurs bénéfices potentiels et leurs limites, permettant ainsi d'apporter des alternatives valables pour le futur. Comme leurs prédécesseurs, les antibiotiques, l'utilisation de ces alternatives se fera via un programme incluant différents produits dont l'effet synergie ainsi que les modes d'actions seront connus et dont la toxicité, les résidus et la résistance microbienne ne seront pas mis de côté.

Mots-clés : Extraits naturels, production animale, mode d'action, bénéfices, analyse de risque.

Introduction

"Let your food be your first medicine" (Hippocrates, 377 BC) was probably the first time that the link was made between nutrition and well-being. This old aphorism has recently come back into fashion in human and animal nutrition over the last few years as the global markets have returned to a more "natural" source. In the animal feed industry, most diets can provide the diverse blend of nutrients needed for growth, maintenance and overall well-being. In some instances, however, the diet formulation alone may not supply adequate amounts of the required nutrients, or the animal may not be in a state to make proper use of it as is the case with ageing, during pregnancy and lactation, or under stress or disease challenge. During the twentieth century, much work had been directed towards elucidating the biochemical structures and physiological roles of many nutritional additives, especially the routine inclusion of in-feed antibiotic growth promoters in pig and poultry diets over the last 50 years. These have proven to be an effective method of both enhancing animal performance and reducing digestive disorders which often presented themselves as scouring, particularly in weaners. At the same time,
increased concerns over food safety, environmental contamination and general health risks have
made "natural" the norm, promoting the trend toward alternative strategies to manage and feed
young animals without reliance on antibiotics. In response to this void in the marketplace, a new
generation of alternative products have vied to take their place, many of whom present more
questions than answers. Whether labelled under the name of "adaptogens", "dietetics",
"neutraceuticals", "neutricines" or "multi-functional additives", these are marketed for their
benefits on animal nutrition. Among these are included the natural plant extracts. Much research
has focused on the specific beneficial effects of the inclusion of these as feed micro-ingredients,
but this is by no means exhaustive. In particular, it remains unclear if a particular action, i.e.
antioxidant, antiseptic, immunomodulator, etc., can be associated with a specific molecule, and
this is complicated even more one active substance may have multiple actions. In some cases,
while searching for the "magic bullet", what we may be end up with is more of a "dietary machine
gun". Nonetheless, the elucidation of well-defined modes of action will provide the scientific basis
for establishing the efficacy and safety of these additives so as to develop a long-term strategy
for their positioning with respect to specific diet formulations and other feed supplements. Only
then will the industry and consumer feel confident about the potential of these products and
embrace them as part of standard practice, as once they did with the antibiotic growth promoters.

**Historical overview**

One generation of productivity enhancers includes additives based on natural plant extracts
and their intrinsic active principles. The use of plants and their respective extracts dates back
some thousands of years to the ancient Egyptians, Chinese, Indians and Greeks. Even
Christopher Columbus discovered America while looking for a shortcut to many of the herbs and
spices found today in practically everyone's cupboards in one form or another. In addition, many
natural extracts have provided the basis for modern medications, such in the case of digoxin from
the digitalis plant, ephedrine from the Chinese herb ma huang, and common aspirin from willow.
Clearly, we can learn from those who came before us, but the empirical knowledge of plants has
risen to another level due in large part to modern technologies which have led to the systematic
isolation and characterisation of active principles contained in these vegetal sources. As
summarised in Table 1, many of today's extracts have beneficial multi-functional aspects which
derive from their specific bio-active components. This represents only a fraction of the phytophagenic
elements known to man, however. To date, more than 10,000 species of plants known in the
modern world remain undiscovered, and 90% of the molecules which account for many of the
actions still remain a mystery! Through the integration of current know-how, these molecules will
become easier to find, identify and characterise. Furthermore, information regarding dose-
response, metabolism, toxicity and maximum residue levels (MRL) should also be accumulated
with the intent of establishing an international database of information available to feed experts
and consumers alike.

**Back to basics**

**Relating structure to function**

Plant extracts contain a vast source of different molecules which have intrinsic bio-activities on
animal physiology and metabolism. One example are the phenolic compounds, a diverse series of
either single (simple phenolic) or multiple (polyphenolic) aromatic rings differing principally in their
respective side chains (Fig. 1). In this sense, the phytophagenic active components share something
in common with organic acids, which also represent a non-homologous group, varying in the
numbers of carboxy groups, hydroxy groups and carbon-carbon double bonds at the molecular
level. These structural differences confer different stabilities of polyphenolic compounds over a
standard pH range of 3-11. A funded project is ongoing at TEKTRAN of the United States
Department of Agriculture Research Service to investigate the family of phytophagenic phenolic
compounds. In this program, series of structurally well-defined phenolic compounds are being
tested for their stability coefficients by changes in the ultraviolet absorption spectra of the
phenolic compounds left standing in different pH buffers for several periods up to 72 hours. The
results of this screening have suggested that the stability of the phenolic compounds strongly
depended on the structure of the compound, the pH and length of storage buffer time (Friedman
and Jurgens, 1999).
Table 1. Summary table representing common plant extracts, their utilised parts, main active substances and reported properties (Richard, 1992; Charalambous, 1994)

<table>
<thead>
<tr>
<th>Vegetal form</th>
<th>Utilised parts</th>
<th>Main compounds</th>
<th>Reported properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic spices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutmeg</td>
<td>Seed</td>
<td>Sabinene</td>
<td>Digestion stimulant, antidiarrhoeic</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Bark</td>
<td>Cinnamaldehyde</td>
<td>Appetite and digestion stimulant, antiseptic</td>
</tr>
<tr>
<td>Clove</td>
<td>Cloves</td>
<td>Eugenol</td>
<td>Appetite and digestion stimulant, antiseptic</td>
</tr>
<tr>
<td>Cardamom</td>
<td>Seed</td>
<td>Cineol</td>
<td>Appetite and digestion stimulant</td>
</tr>
<tr>
<td>Coriander</td>
<td>Leaf, seed</td>
<td>Linalol</td>
<td>Digestion stimulant</td>
</tr>
<tr>
<td>Cumin</td>
<td>Seed</td>
<td>Cuminaldehyde</td>
<td>Digestive, carminatif, galactague</td>
</tr>
<tr>
<td>Anise</td>
<td>Fruit</td>
<td>Anethol</td>
<td>Digestion stimulant, galactagogue</td>
</tr>
<tr>
<td>Celery</td>
<td>Fruit, leaf</td>
<td>Phtalides</td>
<td>Appetite and digestion stimulant</td>
</tr>
<tr>
<td>Parsley</td>
<td>Leaf</td>
<td>Apiol</td>
<td>Appetite and digestion stimulant, antiseptic</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>Seed</td>
<td>Trigonelline</td>
<td>Appetite stimulant</td>
</tr>
<tr>
<td>Pungent spices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsicum</td>
<td>Fruit</td>
<td>Capsaicin</td>
<td>Antidiarrhoeic, anti-inflammatory, stimulant, tonic</td>
</tr>
<tr>
<td>Pepper</td>
<td>Fruit</td>
<td>Piperine</td>
<td>Digestion stimulant</td>
</tr>
<tr>
<td>Horseradish</td>
<td>Root</td>
<td>Allyl isothiocyanate</td>
<td>Appetite stimulant</td>
</tr>
<tr>
<td>Mustard</td>
<td>Seed</td>
<td>Allyl isothiocyanate</td>
<td>Digestion stimulant</td>
</tr>
<tr>
<td>Ginger</td>
<td>Rhizome</td>
<td>Zingerone</td>
<td>Gastric stimulant</td>
</tr>
<tr>
<td>Aromatic herbs and spices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garlic</td>
<td>Bulb</td>
<td>Allicin</td>
<td>Digestion stimulant, antiseptic</td>
</tr>
<tr>
<td>Rosemary</td>
<td>Leaf</td>
<td>Cineol</td>
<td>Digestion stimulant, antiseptic, antioxidant</td>
</tr>
<tr>
<td>Thyme</td>
<td>Whole plant</td>
<td>Thymol</td>
<td>Digestion stimulant, antiseptic, antioxidant</td>
</tr>
<tr>
<td>Sage</td>
<td>Leaf</td>
<td>Cineol</td>
<td>Digestion stimulant, antiseptic, carminatif</td>
</tr>
<tr>
<td>Bay laurel</td>
<td>Leaf</td>
<td>Cineol</td>
<td>Appetite and digestion stimulant, antiseptic</td>
</tr>
<tr>
<td>Peppermint</td>
<td>Leaf</td>
<td>Menthol</td>
<td>Appetite and digestion stimulant, antiseptic</td>
</tr>
</tbody>
</table>

Fig. 1. Chemical structures and molecular weights for common plant active substances. (A) anethol, MW 148.2; (B) cinnamaldehyde, MW 132.2; (C) eugenol, MW 164.2; (D) thymol, MW 150.2.
Principal components analysis (PCA) performed at the Department of Microbiology at the University of Auvergne Faculty of Medicine, Clermont-Ferrand, France, has also been used to study the properties of a preliminary screening panel of plant extracts. The pH distribution of this panel (data not shown) ranged from pH 4.3 (cinnamon extract) to pH 6.7 (ginger extract). This analysis continues to support the theory that all extracts are not created equal, but are representative of a group of structurally and chemically distinct molecules.

In addition, previous work has shown that, like organic acids, these chemical properties may correspond structurally to the incidence of polar groups, the number of double bonds, molecular size, and solubility in non-polar matrices. This, in turn, may dictate the activities observed in vitro: (i) antiseptic (Hammer et al., 1999); (ii) antifungal (Apisariyakul et al., 1995); and (iii) antioxidant (Aeschbach et al., 1994); as well as in vivo: (i) stimulation of feed intake (Petit et al., 1993); and (ii) stimulation of digestive enzymes (Platel and Srinivasan, 1996); and still others.

Natural plant extracts show structure-specific MIC\textsubscript{50} spectra

The antiseptic effects of natural plant extracts remains uncontested over time. The scientific literature is filled with past and recent work supporting the minimum inhibitory concentration (MIC\textsubscript{50}) and minimum bacteriocidal concentration (MBC\textsubscript{50}) of these substances. What has remained unclear is the relationship between structure of the active substances found in plant sources and their effects. Preliminary work carried out in collaboration with the University of Auvergne Department of Microbiology has confirmed that different extracts do indeed have different MIC\textsubscript{50} spectra when screened against a diverse bacterial panel (Table 2).

Table 2. MIC\textsubscript{50} values for selected plant extracts against a panel of gram-positive, gram-negative and anaerobic bacteria (performed in collaboration with the Department of Microbiology, University of Auvergne, Clermont-Ferrand, France)

<table>
<thead>
<tr>
<th>MIC\textsubscript{50} Values</th>
<th>E. coli H7:O157</th>
<th>S. typhimurium</th>
<th>C. coli</th>
<th>C. perfringens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsicum extract</td>
<td>NE†</td>
<td>NE</td>
<td>NE</td>
<td>50</td>
</tr>
<tr>
<td>Cinnamon extract</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Clove extract</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Garlic extract</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>100</td>
</tr>
<tr>
<td>Oregano extract</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Romarin extract</td>
<td>50,000</td>
<td>50,000</td>
<td>50,000</td>
<td>50,000</td>
</tr>
</tbody>
</table>

†NE = No effect.

From these studies, romarin showed very little effect against gram-negative micro-organisms. Cinnamon, clove and oregano showed moderate and wide-spectra efficacy against the panel. The spice extracts, capsicum and garlic, showed specific efficacy against Clostridium sp. alone. These differences in efficacy at the in vitro level can be clearly linked to the level of their active substances, with purity of the plant extract playing a critical role (C. Forestier, University of Auvergne, pers. comm.). While their in vitro effects are well-documented, the mode of action by which these substances exert their effect on bacteria is current being investigated. On-going work seems to confirm that these substances, like antiseptic agents in general, exerts its effects on by disrupting the microbe at the cellular membrane. The effect of natural plant extracts on the hydrophobicity of an E. coli strain of post-weaning pig origin was determined by the salt aggregation test, a measure of the aggregative (binding) properties of strains of bacteria. The preliminary panel included extracts from St. John's wort, cassia, cinnamon and thyme which showed MIC\textsubscript{50} values at 250 p.p.m. or more on E. coli sp. The results shown indicate a strong increase (40-60%) in hydrophobicity of the microbial species by the addition of St. John's wort and cassia. Cinnamon and thyme showed more moderate effects (Table 3). These hydrophobicity differences correlated strongly with the MIC\textsubscript{50} values for the extracts investigated (data not shown). Therefore, one of the probably and potentially important action mechanisms of certain plant extracts may be their ability to influence the surface characteristics of microbial cells and thereby their putative virulence properties. This has interesting implications in the animal gut where the adhesion of microbes on host cells is of decisive importance in the development of gram-negative microbe-induced infections, and which is influenced strongly by the surface
hydrophobicity of the microbial cell (Mbawambo et al., 1996).

The role of complementary in vitro modelling techniques

The feed industry, and young animal breeding in particular, is shifting into a phase of intensified research and development. Few companies, however, have the adequate manpower or overhead to put into place and follow long-term research programs, while on the other hand, public institutions often have difficulty finding the financial support necessary for project funding given the dwindling government-sponsored funding programs available. While this has become the norm in human nutrition and medical research, the possibilities are just beginning in the animal feed industry for a pooling of resources from the public and private sectors to work jointly toward one or more common objective(s).

Table 3. Hydrophobicity of E. coli (suis strain 4596) in the presence of St. John's wort, cassia, cinnamon or thyme. Values expressed as percentage aggregation by the Salt Aggregation Test

<table>
<thead>
<tr>
<th>Aggregation</th>
<th>St. John’s wort extract</th>
<th>Cassia extract</th>
<th>Cinnamon extract</th>
<th>Thyme extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (suis 4596)</td>
<td>58%c</td>
<td>42%b</td>
<td>23%a</td>
<td>28%a</td>
</tr>
</tbody>
</table>

Values on the same line with different postcripts are significantly different.

One example where this may be most easily realised in the immediate future is in the development and validation of complementary in vitro systems modelled after various aspects of animal behaviour and physiology. There is a growing need for the development of systems which will be able to complement the information obtained through classical in vivo animal testing, while adding a dimension toward understanding small yet significant mechanisms, benefits or constraints which may be remain hidden due to internal or external variability. In one case, new feed additive product R&D has been and will continue to be one area where in vitro modelling operates at its best. Probably the greatest advantages of these models lie in qualities that are not so obvious. Trials with most systems are neither time nor labour intensive, contributing to costs running only a fraction of the cost of comparable live animal trials. There are also advantages in the case of animal welfare considerations, as no live animals are directly involved. And even though these are not the true animal itself, in function they can come very close to the real thing in certain aspect.

The value of these models is seen as a tool for visionary feed additive companies whose goal is to aid nutritionists and feed compounders in the difficult, time-consuming challenge of evaluating products and their combinations within a range of diet formulations and production practices. Using these models, we have been able to screen a “library” of plant extracts to investigate their effects on many aspects from bioavailability, normal digestion and gut flora mechanics, environmental and animal impact as it pertains to traceability and residues.

Establishing reproducible methods of detection for new alternative products

Traceability and residues are probably two of the “hottest” topics of discussion in today's global feed industry. The need to be able to follow the course of a product and its active ingredients from start to finish is seen as primordial. Reliable detection techniques such as those found in most analytic laboratories, such as GC (gas chromatography), LC (liquid chromatography), MS (mass spectrophotometry) and HPLC (high-pressure liquid chromatography) as well as their combinations, are necessary to attain this end. Early work has shown that the phenolic compounds (Fig. 2) can be identified and quantified, with detection systems reaching the sensitivity levels of picograms of material depending on the system in place. (For reference, 1 picogram is the equivalent of one billionth of a gram.) These analytical techniques will be vital for implementing programs of feed traceability as well as establishing minimum residue levels (MRL) as part of a carcass, egg and milk evaluation programs for these categories of products.
In vitro intestinal epithelium cell cultures

The first step in traceability is in the determination of what level of the compound crosses the intestinal epithelium. If the substance in question and its respective metabolites remain intraluminal and are finally excreted in the urine or feces, there is little need to check for its deposition in internal depots. In this regard, immortalised epithelium cell systems such as the Caco-2 cell line, derived from a human colon adenocarcinoma, is seen most similar morphologically and functionally to intestinal epithelial cells (Fig. 3). Indeed, there is an extensive database of several thousand scientific publications relating to these cells making them an extremely well-characterised research and development tool for many areas of scientific research. Their use has been quite extensive in the area of pharmacology, especially since there is a high degree of correlation between the permeability to pharmacological agents and the absorption of these same compounds in live subjects. This demonstrates only one value of the system in predicting potential bioavailability and absorbability of active compounds. In addition, it can be used to screen for detrimental effects of novel compounds upon ingestion. We have seen the exciting potential of this system in the traceability, safety and toxicity studies in the elucidation of plant extracts and their intrinsic active substances. Preliminary screening of panels of extracts and their active components have shown that these substances can be traced to different intracellular as well as transcellular destinations (data not shown). These, in turn, may be dictated by the cellular sorting signals found deep in their molecular structure.

Fig. 2. Sample extract representing 50 picograms of zingerone, from Zingiber officinale Roscoe, analysed by electrospray ionisation in LC-MS.

Fig. 3. Schematic representation of Caco-2 intestinal cell culture system illustrating its two
interfaces: apical (luminal) and basolateral (blood). Substances added at the apical side are monitored over time along the cell transport system.

Dynamic monogastric intestinal model

Another tool which is representative of the in vitro systems being developed in the public sector is the monogastric intestinal model, developed by Drs. Robert Haavenar and Mans Minekus at the TNO Institute in Zeist, Netherlands. Briefly, this accurate "mechanical" model provides continuous monitoring of the natural parameters of digestion and its by-products. This can in turn determine the traceability of ingested feed components as well as their possible effects on the animal and their impact on the environment (Kamel, 1999). Certainly any type of digestion modelling has its limits, but this model has provided clear in-roads into the modes of action of various plant extracts and their possible usage in combination with other alternatives such as organic acids. Feed with the inclusion of certain plant extracts have demonstrated effects in the gastric compartment parallel to those observed with specific organic acids (Fig. 4). Specifically, some extracts exert an acid-sparing effect when included in the feed whose basic pH nature could stress the animal's gastric acid production which has as its consequences poor feed digestibility and the source for pathogenic bacterial overgrowth.

![Liberation of gastric acid by the inclusion of a combination of natural plant extracts](image)

**Fig. 4.** Acid-sparing effect from the inclusion of natural plant extracts in a post-weaning pig diet.

The inclusion of plant extracts in the in vivo situation

For many years, feed additives have been used particularly in young animal diets where digestion problems at weaning lead to scouring and a growth "check". The slow, uneven acidification of the contents of the stomach in the young animal plays a major role in the classic over-proliferation of *E. coli*, which then leads to enterotoxaemia in weaned piglets. The nutritional effectiveness of organic acids in piglet and broiler breeding has permitted it to become a standard staple in young animal diets, but the raw material changes and the evidence for "acid-resistant" micro-organisms such as *E. coli H7:O157* have led many feed industry diet experts to use acid blends or the combination of acids with other alternatives, such as enzymes or probiotics. Early evidence into the modes of action of certain plant extracts and phytogenic active substances indicate that a synergism may occur between this class of compounds and organic acids. Development using many of the before-mentioned laboratory and in vitro modelling systems have shown promise in optimising the actions of these products alone and in combination with each other. A series of post-weaning pig trials is underway to confirm the promising combinations studied up until now. One such trial (Table 4) was conducted in a commercial farm in Switzerland in pigs weaned at 21 days of age. The results show the benefits of the inclusion of a precise combination of plant extracts on both daily weight gain (DWG, +7.3% compared to control) and feed conversion (FCR, +5.9 compared to control). The addition of a blend of organic acids to the plant extracts enhanced the observed effects even more dramatically, as well as having a significant effect on the incidence of diarrhoea. The evidence that this strategy may be useful in reducing the stress associated with early-weaning programs is currently being studied at several levels to determine the underlying mechanisms in vivo.
Conclusions

Natural plant extracts represent one of a myriad of alternatives in response to the void created from the ban on antibiotic growth promoters in Europe. These substances have been in use since the beginning of recorded history, but little is known about the mechanisms which lead to the benefits seen both in man and animal. Furthermore, it will be up to the feed additive supplier to be responsible for a "checklist" of issues surrounding these components, such as: (i) composition identification; (ii) technical and zootechnical efficacy; (iii) toxicity analysis; (iv) feed traceability; (v) residue analysis; and (vi) exposure and handling risks. Even though this will be an arduous task for many, it will represent a responsible, informed approach within the feed industry and towards the consumer.

Table 4. Experimental means for daily feed intake (DFI), daily weight gain (DWG), feed conversion ratio (FCR) and diarrhoea scores of post-weaning pigs fed from 21 to 42 days on diets either containing natural extracts or a combination of natural extracts and organic acids

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Negative control (no additive)</th>
<th>Natural extracts XT</th>
<th>Natural extracts XT + organic acids AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFI (g/d)</td>
<td>491</td>
<td>503</td>
<td>530</td>
</tr>
<tr>
<td>DWG (g/d)</td>
<td>292b</td>
<td>315a</td>
<td>335a</td>
</tr>
<tr>
<td>FCR</td>
<td>1.68b</td>
<td>1.58a</td>
<td>1.52a</td>
</tr>
<tr>
<td>Diarrhoea score</td>
<td>39.0b</td>
<td>33.8ab</td>
<td>28.5a</td>
</tr>
</tbody>
</table>

a,b Values on the same line with different postcripts are significantly different.

References


