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Humic Acid Substances in Animal Agriculture

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Abstract: Humic acids (HA), a class of compounds resulting from decomposition of organic matter, particularly plants are natural constituents of drinking water, soil and lignite. It inhibit bacterial and fungal growth, thus decrease levels of mycotoxins in feed. Stress management, immune system, anti-inflamatory activity, antiviral properties as well as prevention of intestinal diseases, mainly diarrhoea in humans and animals are described as its beneficial effect. The use of HA and related products in feed improved gut health for better nutrient utilization as well as improved the health status by working against pathogens by developing immunity. Routine use of HA in feed improved growth of broilers by increasing digestion of protein and trace element utilization but a few researches has been conducted in this area. However, also contradictory findings in piglets are described. Most of the literature found is from companies but scientific articles are rather limited. There are also strong limitations in the knowledge of HA uses as feed additive for growth of other species of animal. It is really difficult to compare the actual effects of HA preparations due to different sources and nature as well as because rearing of animal in different region of the world varies as climatic conditions and aspects. So, bio-effect of HA product depends on specification.

Key words: Humic acid, animal and agriculture, immune system

Introduction

Antimicrobial feed additives are world wide used so far in animal husbandry to improve the economy and ecology of animal production by increasing growth rate, decreasing feed expenditure per gain and diminishing the risk of disease (Hays, 1981; Gropp *et al*., 1992). But the unavoidable spread of bacterial resistance and cross-resistance to antibiotics used in veterinary and human therapy (Barton, 1998; Khachatourians, 1998) increasingly considered as a hazard, therefore the approval of antimicrobial growth promoters will be phased out by EU legislation by the end of 2005. Among many alternatives Humic acids (HA) are described. There are literature states that it has growth related effect as well as health protection capacity by changing some physiology and developing immunity in different species of animal. However, also contradictory findings in piglets are described (Schuhmacher and Gropp, 2000). Most of the cases different companies claims the HA substances as beneficial for the production of farm animal. So, it is important review the literature to draw an actual picture in relation to animal agriculture.

Organic matter in the soil exists in 3 different forms: (1) Living plant and animal matter, (2) Dead plant and animal matter and (3) decomposed plant and animal matter (humic substances). So, humic substances are the most common forms of organic carbon in the natural environment.

Concept of humic acid

Most humic substances are chemically attached to inorganic components (clay and oxides), and a smaller part gets dissolved in the solutions of the soil, particularly under alkaline conditions. An important feature of humic substances is that they can combine with metal ions, oxides and clay minerals to form water soluble or insoluble complexes and can interact with organic compounds such as alkenes, fatty acids, capillary-active substances and pesticides.

Farmers use humates to accelerate seed germination and improve rhizome growth (Humet Product Documentation and Technical Information, 1999). These materials are able to stimulate oxygen transport, accelerate respiration and promote efficient utilization of nutrient by plants (Visser, 1987; Österberg and Mortensen, 1994). These observations prompted scientists to study the specific properties of humates and their possible benefits in improving health and well being of humans and animals. Several humic substances have been identified.

Humus: This is the fraction of humic substances that is not soluble in water at any pH value. These substances have the greatest molecular sizes, as their molecular weights can be around 300,000 Dalton. The oxygen content in this substance is the lowest and falls in the

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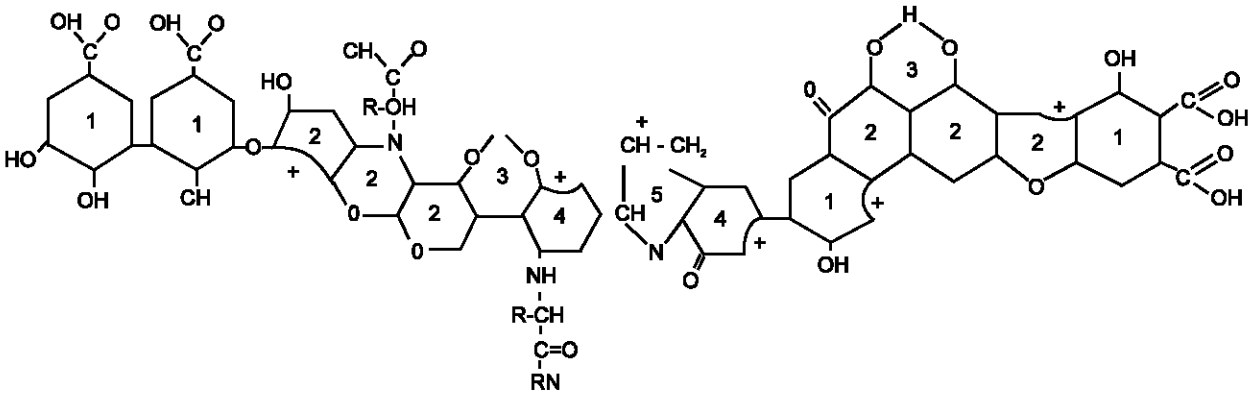

Fig. 1: Oxidized HA molecule

range of 32-34 %, while the nitrogen content is the highest, being around 4 %. Because of the high molecular weight, the negative surplus charge on their surfaces is insufficient for peptising the macromolecules even at strongly alkaline pH, and so their mobility in the soil is insignificant when in a coagulated state.

Humic acids: Humic acids are humic substances not soluble in water under acid conditions (below pH 2), but become soluble at a greater pH (HuminTech, 2004). Humic acids are soluble in dilute alkaline solutions and precipitate as soon as the solution becomes slightly acidic. These substances have medium molecular size and their molecular weight is around 5,000 to 100,000 Dalton. Oxygen represents 33-36 %, while nitrogen represents 4 % in this substance. Because of their medium molecular size, sufficient negative surplus charge on their surfaces for peptising the macromolecules will occur only in a more alkaline medium with a pH over 8 and thus their mobility in the soil is limited in neutral acidic-alkaline conditions.

Fulvic acids: Fulvic acids are soluble under all pH conditions. Those dissolve in dilute alkaline solution and will not precipitate even if the solution turns slightly acidic. These substances have the lowest molecular size, as their molecular weight is around 2,000 Dalton. This is the material with the highest oxygen content (around 45-48 %) and the lowest nitrogen content (less than 4 %). Because of their low molecular weight their surface negative surplus charge is sufficient to peptise the macromolecules even at neutral or slightly alkaline conditions resulting in significant mobility in the soil.

Phenolic acids: These substances are not defined based on solubility but identified as a component of humic substances.

Chemical structure of humic acid

Bio-Liquid Complex (Bio Ag Technologies International) derived from a type of leonardite (highly oxidized form of organic matter) differs from their theoretical formula because a part of its chemical structure has been oxidized. These broken bonds create places on the molecules where micronutrient ions can be absorbed. The oxidized sites give the entire molecule a negative charge enabling it to absorb micronutrients (Fig. 1). In the past these compounds have been largely overlooked because of the unavailability of adequate and sensitive analytical methods. A chemical structure for the basic skeleton of HA is based on alkylbenzenes,-napthhalenes and -phenanthrenes.

Soluble HA is available as either potassium humates or sodium humates (ie HA is only soluble in an alkaline base). Potassium humates are the product of choice for the soil because extra sodium is rarely required. Sodium humates are preferable for animals as sodium is an important inorganic electrolyte for animal.

Bioavailability and Composition of Humic Acid Products

A sample of Humisolve-R (Faust Bio-Agricultural Services) containing 73 % HA shows the following bioactive organic groups (Faust, 1998).

a) 3.32 % carbonyl, carboxyl and quinone groups
b) 28.1 % of phenol hydroxyls and nitrogen-containing aromatic groups
c) 7.78 % of aromatic and heterocyclic compounds
d) 44.7 % of protonated aromatic carbons
e) 16.06 % of methyl and methylene groups

Total bioactive organic group (a-d) in that sample of humate was 83-94 %. The activity had been confirmed by bioassay that had connected those bioactive organic chemicals to the humates on plants and animal cells (Faust, 1998).

Certified composition of HA/HuminFeed produced by Humintech GmbH, Heerdter Landstr. 189/D, D-40549 Düsseldorf, Germany, is (1) Water 14,50 %; (2) Ash (DM basis) 26,00 %; (3) Humic acids (DM basis) 74,00 %; (4) Sodium 8,9 %;(5) pH (in 10 % solution) 9 to 10. In a case study by Enviromate TM, 2002, the HA material contained

Table 1: Effect of dietary Farmagülatör DRY™ Humate on performance of male broilers (Kocabagli *et al.*, 2002)

Dietary regimes	Hatch weight	Body weight (g) (21 d)	Body weight (g) (42 d)	Feed:gain (g:g) (0-21d)	Feed:gain (g:g) (22-42)	Feed:gain (g:g) (0-42)	Overall mortality (%)
NAFH[1]	45.6	758	2346[b]	1.68	2.14[a]	1.99	8.0
$FH_{(0-21)}$[2]	45.5	735	2394[ab]	1.74	2.03[ab]	1.95	1.0
$FH_{(22-42)}$[3]	44.9	749	2451[a]	1.67	1.99[b]	1.89	5.0
$FH_{(0-42)}$[4]	44.6	737	2428[ab]	1.71	1.99[b]	1.92	7.0
SEM	0.31	7.9	25.9	0.039	0.037	0.032	2.03
P- value	0.144	0.140	0.029	0.545	0.0416	0.2522	0.114

[1]No added Farmagulator DRY™ Humate; [2]Farmagulator DRY™ Humate provided from 0 to 21 d (2.5 kg/ton feed); [3]Farmagulator DRY™ Humate provided from 22 to 42 d (2.5 kg/ton feed); [4]Farmagulator DRY™ Humate provided from 0 to 42 d (2.5 kg/ton feed)

Table 2: Effect of HA containing diets on live weight, feed conversion efficiency (FCE; gLWG/1000 g feed intake) and mortality of broiler[1]

Code	(1)	(2)	(3)	(4)	(5)	(6)
Humic acid (g kg^{-1} feed)	0	0.3	0.6	1.2	2.4	4.8
Composition of feed						
CP (g/100g air dry sample)	26.20	26,16	26.00	25.70	25.06	23.47
ME (MJ kg^{-1} feed)[2]	12,73	12,69	12,65	12,54	13,09	12,52
Performance of broiler						
Weight /bird on day 7	193.4[a]	193.5[a]	192.0[a]	192.3[a]	185.0[a]	174.2[b]
Weight /bird on day 14	547[a]	541[a]	543[a]	536a	521a	483[b]
Weight /bird on day 35	2408	2369	2335	2355	2310	2301
FCE from 0 to7 days	1011[a]	988[ab]	1020[a]	992[ab]	921[c]	930[bc]
FCE from 0 to 35 days	723[a]	712[ab]	715[ab]	709[ab]	701[ab]	689[b]
Mortality (%)	8.75	5.00	1.25	2.50	1.25	5.00

CP-Crude protein; [1]Research conducted at the Institute of Animal Nutrition, Nutrition Diseases and Diatetics, University of Leipzig, Germany. [2]Calculated from FMV (ME in MJ/kg = g Crude protein x 0.01551 + g Crude Fat x 0.03431 + g Starch x 0.01669 + g Sugar x 0.01301) according to German legal regulations (see Weinreich *et al.*, 2002). [abc]Different superscripts in the same row differ significantly ($p<0.05$)

(1) Crude protein 7.10 %; (2) Ash 8.33 %; (3) Crude fibre 12.50 %; (4) Carbohydrates 51.20 %; (5) Nitrogen 1.14 %; (6) Moisture 8.60 %; (7) Humic acids 42-48 %; (8) Fulvic Acids 12 % of HA. Many other literatures (not mentioned here) showed that the HA composition varies between the HA preparations of different companies but also between the different sources (soil), they were extracted from.

Humic acids as feed additive

Humic acids are not approved as feed additive, but as veterinary drug at EU level although many literatures indicated those as feed additives claiming growth promotion effect.

Better performance by improving nutritive value of feed: In recent years, it has been observed that humates included in feed and water of poultry promotes growth (Bailey *et al.*, 1996; Parks, 1998; Shermer *et al.*, 1998; Eren *et al.*, 2000). Kocabagli *et al.* (2002) studied to use (2.5 g kg^{-1}) of Farmagülatör DRY™ Humate (FH) (Farmavet International) on live performance, carcass weight, and the abdominal fat pad of broilers during different feeding periods (control-without FH), FH from 0-21 day (starter period), FH from 22-42 days (grower period). Feeding FH during the grower period had the most beneficial effect in terms of growth and feed conversion (Table 1). An other study (Eren *et al.*, 2000), compared the effects of dietary humate (Farmagülatör DRY™) supplementation at 1.5 and 2.5 g kg^{-1} feed on broiler performance from 0 to 42 d. Although there was no performance difference at 21 d, the authors found that dietary supplementation of humate at 2.5 g kg^{-1} significantly improved the live weights of broilers at 42d. They also showed that serum Na+ concentration and tibia bone ash of male broilers were significantly elevated when humate was fed at 2.5 g kg^{-1} but not 1.5 g kg^{-1}. Also a recent study conducted by the author at the Institute of Animal Nutrition, Nutritional Diseases and Diatetics, University of Leipzig, Germany indicated that the inclusion of HA during later stage in broiler diet could more beneficial in respect of performance (Table 2).

In 1998, a test was carried out on a wide scale at the Severny pedigree poultry breeding state farm near the town of Bratsk. The poultry losses decreased by 50%, while the live weight in five weeks increased by 30% (Teravita, 2004). The results, shown in the Fig. 2, strongly supported the recent findings of the author

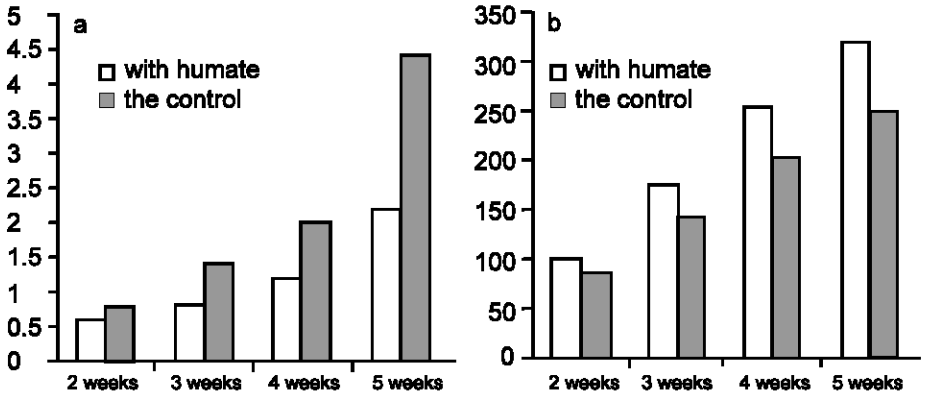

Fig. 2: The effect of the humate supplements on poultry loss (a) and live weight gain in poultry (b).

(Table 2) in respect of mortality but not strong for growth. Conversely Bailey *et al.* (1996) found that feeding 5 g Menefee® humate (MH) kg^{-1} feed (Sundine Enterprises, Inc.) to male broilers did not affect body weight, but improved feed conversion at 35 d. They also reported that dietary MH supplementation increased mortality significantly but also body weight by day 42 in female broilers. In another report with turkeys feeding MH improved weight gain and feed conversions from 8 to 12 wk of age ($P<0.05$), but this response did not persist until 20 wk of age. But, incorporation of humates in the feed of chickens has reduced unspecific deaths by 3 to 5 % (Stepchenko *et al.*, 1991) also supported by Kocabagli *et al.* (2002) and the recent work of the author (Table 2).

Humic acids stabilize the intestinal flora and thus ensure an improved utilization of nutrients in animal feed. This leads to an increase in live weight of the animal without increasing the amount of feed given to the animal (HuminTech, 2004). In the same brochure it is mentioned that diet digestibility as a result of maintaining optimum pH in the gut increases, resulting in lower levels of nitrogen excretion and less odour. Moreover, HA is said to improve protein digestion as well as calcium and trace element utilization.

The HA also leads to increased milk production and increased butterfat percentage in dairy cows, improved feed efficiency, decreased feed costs, reduced fly population and reduced costs for insect control. Furthermore, the weaning weights increased and faster weight gains were observed, while problems with scours greatly decreased (Livestock R. Us, 2003). On the whole, HA should increase the animal's resistance against heat stress.

Calves born from cows that have been fed humates, had a 13.4 % more weight within four months compared to control. The bull-calves that had been fed with humates, had an increase of 21.2 % body weight than control. The haematological data of animals in both humate-fed groups showed increased levels of haemoglobin by 11.5 %, phosphorus by 6.7 %, albumen by 24.3 %, and the β-globulin level increased by 32 %. In broilers feed activated the synthetic phase of albumineous exchange. As a result, there was a 10 % increase in body weight, and an immunity rise by 5-7 % (TeraVita, 2004).

Yasar *et al.*, 2002, concluded that HA caused increased weight gain in rats. The improved weight gain was associated with increased ileal epithelial mass, increased feed intake, improved feed:gain ratio and increased nitrogen retention in rats.

Replacing antibiotic growth promoter: Replacing antibiotic with HA as growth promoter in animal feed does not cause any loss in the performance of animals. On the contrary, performance factors (LWG, FI, FCR and faeces consistency - scour assessment) of animals are considerably improved. The use of HA in animal feed excludes of course the possibility of antibiotic residue or microbial resistance (Humin Tech, 2004). Simultaneously, as a result of a higher food conversion rate and enhanced absorption of nitrogen by the animal, nitrogenous wastes and odour are reduced. It was also concluded by Ceylan and Ciftci, (2002), that HA would be an alternative to antibiotic growth promoters in broiler diets.

Health value of humic acid substances

Scientists at the Drepropetrovish Agricultural Institute in Moscow revealed humate as harmless with respect to blood, cardio-vascular system, endocrine system and other vitally important organs using patho-histological and histo-chemical methods. The toxicity of naturally occurring HA is remarkably low (Thiel *et al.*, 1981). An LD_{50} of 0.536g kg^{-1} bw can be considered as confirmation of the harmlessness of humate (Lotosh, 1991). Current repeat toxicity studies in rodents indicated total safety at levels up to 50 mg kg^{-1} body weight (Laub, 1998b).

Humic acids should inhibit pathogenic bacterial growth and growth of moulds, thus decreasing levels of

Table 3: Relative organ weight (liver, spleen and Bursa fabricii) in percent of body weight of humic acid fed broiler

Code	(1)	(2)	(3)	(4)	(5)	(6)
Humic acid (g kg^{-1} feed)	0	0.3	0.6	1.2	2.4	4.8
Liver	2.21	2.34	2.20	2.31	2.22	2.19
Spleen	0.08	0.11	0.10	0.10	0.08	0.09
Bursa fabricii	4.22	4.46	3.23	4.15	2.92	3.26
Thyroid gland	0.009	0.012	0.011	0.011	0.011	0.010

NB: Experiment conducted for a period of 35 days at the Institute of Animal Nutrition, Nutrition Diseases and Dietetics, University of Leipzig, Germany. No significant differences observed among the treatments for different parameters ($p>0.05$)

Table 4: Morphometric parameters of thyroid gland of chicks offered different levels of humic acid in feed

Code	(1)	(2)	(3)	(4)	(5)	(6)
Humic acid (g kg^{-1} feed)	0	0.3	0.6	1.2	2.4	4.8
No. of follicles/location*	19.64^{a}	17.34^{ab}	18.38^{ab}	12.68^{b}	17.91^{ab}	18.04^{ab}
Diameter of follicles (µm)	64.41^{a}	70.58^{ab}	71.1^{ab}	84.47^{b}	70.30^{ab}	69.96^{ab}
[1]Height of epithelia (µm)	4.23	4.35	4.23	4.59	4.11	4.00
[1]Length of nucleus (µm)	5.31^{a}	5.11^{ab}	4.96^{ab}	4.72^{b}	4.74^{b}	4.74^{b}
[1]Width of nucleus (µm)	2.80	3.00	3.00	2.95	2.76	2.65
No. of hyperplasia	1.05^{a}	0.79^{ab}	0.67^{ab}	0.59^{ab}	0.51^{b}	0.75^{ab}
FEI	15.38	16.33	16.98	18.66	17.36	17.80

*(367.53X285.75 µm); NB. Ten birds per group and 11 location of each slide from right lobe were considered; [1]Ten replicates were considered per location of the slide from a bird and 110 replications per bird/slide. Follicle epithelium index (FEI) = Follicle diameter (µm)/Epithelial height (µm). [abc]Different superscripts in the same row differ significantly ($p<0.05$)

mycotoxin, which should lead to improved gut health (Humin Tech, 2004). Dermal, oral or subcutaneous application of HA leads to inhibitory effects on inflammation. The ability to inhibit inflammation is believes to be related with the flavonoid groups contained in HA.

Humic acids are able to form a protective film on the mucous epithelia of the gastro-intestinal tract against infections and toxins (Kühnert *et al.,* 1991). The macro-colloidal structure of HA ensures a good shielding on the mucous membrane of the stomach and gut, the peripheral capillaries and damaged mucous cells. As a result of this process, the resorption of toxic metabolites is reduced or fully prevented, especially after infections, in case of residues of harmful substances in animal feed or when it is switched to new feeds. Furthermore, HAs also help to prevent excessive loss of water via the intestine (HuminTech, 2004). Those are used in horses, ruminants, swine and poultry at an oral doses level of 500 to 2000 mg kg^{-1} bw for the treatment of diarrhoea, dyspepsia and acute intoxications.

There are some parameters indicating physiological benefits due to HA and related products, they are given below.

Blood parameters: For humans 100-300 mg kg^{-1} bw has no effect on bleeding time, clotting time, thrombin time, plate count, or induced platelet aggregation (Malinowska *et al.,* 1993). Red blood cells (RBC) and haemoglobin level remained on normal levels under the influence of humate in comparison with control (Lotosh, 1991). Literature has indicated that the RBC was capable to carry more oxygen in presence of humate. This additional oxygen causes feelings of euphoria, similar to hyperventilating, during the first few days of taking humate. Healing of injuries, as a result of additional oxygen, is much quicker. Cutting horses have ankle inflammations frequently from their rigorous training programs. Healing times for these injuries have been reduced by the usage of humates. According to Dabovich *et al.,* 2003, a HA product Promax has nutriceutical properties in that it stimulates neutrophil activity which may protect against bacterial pathogens and reduce mortality during acute bacterial infection.

Mineral transfer: Humic acids act as dilator increasing the cell wall permeability. This increased permeability allows easier transfer of minerals from the blood to the bone and cells. Calcification of a bovine implant was improved by 16% (Kreutz and Schlikekewey, 1992). There are also changes in intracellular divalent calcium levels (Yang *et al.,* 1996). Hoewever, literature also reports binding of iodine from foods (Summers *et al.*, 1989) so that antithyroideal effects could be supposed (Seffner *et al.,* 1995). But reverse concluded by (Huang *et al.,* 1994) that the HA do not induce goiter, but they may enhance the goitrogenic effect of low iodine. From a recent study by the author at the Institute of Animal Nutrition, Nutrition Diseases and Diatetics, University of Leipzig, Germany indicated the absence of goiter genic effect in broiler showing lack of dose related effects on visceral organs and histomorphometric parameters of thyroid gland (Table 3 and 4). Just as fulvic acid carries life-sustaining minerals to the body also captures and

removes toxic metals from the body. Fuchs *et al.,* 1982, indicated that the HA had differentiated effects upon trace elements in laboratory rat. Plasma iron levels were hardly affected, while copper and zinc levels were initially suppressed with a tendency for recovery after 60 days.
Seffner *et al.*, 1995, could show that small amounts of HA given to the rat for 8-14 weeks result in histological signs of goitre and trace it back to a reduced iodine availability in the intestine. So, it is contradictory of the recent findings of the author because (Table 3 and 4) Seffner *et al.*, 1995, applied to drinking water, were comparable to our 2.4 g kg^{-1} feed. The rat diet contained (by certificate of the producer) 0.9 mg I kg^{-1}. Authors experimental diet contained 2.5 mg added I kg^{-1} feed. Both dosages were quite above the requirement or the allowance data. Probably, (i) high iodine of diet inhibited the potential occurrence of goitre, (ii) the method was too rough to detect the initial stage of iodine deficiency, and (iii) length of the feeding trial was to short.

Stress management: Literature reports that humates reduce the production of stress causing hormones. This has been cumulated from animal behaviour, in particular from calves first entering the arena. Animals on humate are less affected by the outside stimulus of the crowds or confining areas of the arena. This effect has also been noted on sheep, horses, cattle and hogs. In dairy operations, those animals not on humate aggressively eat their rations while humate animals leisurely graze (Enviromate, 2002).

Microbial interaction: In soil tested for microbial activity, levels increased 400 to 5000 times with the addition of 300 ppm humate into the soil. Humates added to feed stimulate the microbial growth and the extent can be quite large depending upon the species, the culture medium, and the environment (Huck *et al.,* 1991). Species for which natural humic substances have been shown to be inhibitory include *C. albicans, Ent. cloacae, Prot. vulgaris, Ps. aeruginosa, S. typhimurium, St. aureus, St. epidermidis, and St. pyogenes* (Riede *et al.,* 1991). It seems that within the body, humates stimulate the "good" microbes while suppressing the "bad" microbes.
Testing of milk during field trials often indicates an increase of microbes in the milk, an indication to the dairyman of impending mastitis. As a result of feeding humates, mastitis cases within the milking herd dropped from an average of 3 to 4 cases daily to 4 cases in a month (Mosley, 1996). Additional confirmation of reduction of mastitis was observed in lactating female goats.

Immune system: By improving immune functions in the animal, HA are able to reduce the incidence of diarrhoea and other digestive upsets to a considerable extent as well as to improve the animal's defences against pathogens such as *E. coli* (HuminTech, 2004). According to CVMP, 1999, the intramuscular injection of the HA sodium salt (1 mg kg^{-1} bw) to rabbits had no effects on haematological parameters and the glucose concentration in blood, but affected the albumine/globuline ratio in plasma (marked increase of the β-globulin fraction). Pukhova *et al.* (1987) found that sodium humate increases the lifespan of mongrel rats exposed to lethal doses of cobalt radiation.

Anti-inflammatory properties: Humic acids isolated from peat exhibited significant efficacy for adhesions when tested on female rats that had standardized lesions placed on both uterine horns and the peritoneum of the anterior abdominal wall (Yang *et al.,* 1996). According to Kühnert *et al.,* 1982 the humic substances, including peat and sodium humates, are known to exhibit anti-inflammatory properties. Not only does the humate relieve from inflammation, it has been shown to bond to the collagen fibers to aid in repair of damaged tendons and bone. Tendon strength has been shown to increase by as much as 75% (Iubitskaia and Ivanov, 1999; Kreutz and Schlikekewey, 1992).

Anti-viral properties: Humates are effective media additives for the production of antibiotics in the soil (Huck *et al.,* 1991). Humic substances have long been known to exhibit antiviral properties in particular against rhinoviruses (Enviromate, 2002). Viral pathogens for which soil-extract materials have been shown to be effective include in particular Coxsackie virus A9, herpes simplex virus type 1 and 2 (Schiller *et al.,* 1979; Thiel *et al.,* 1981; Thiel *et al.,* 1977; Laub 1998a; Laub 1998b, and Knocking, 1991), human immunodeficiency virus (HIV) (Laub 2000; Laub, 1995), influenza type A and B (Laub 2000 and Enviromate, 2002), as well as other respiratory tract infections (Schultz, 1965; Knocking, 1991 and Jankowski *et al.,* 1993).
In earlier times, HAs have also been employed as veterinary medicine therapy successfully employing peat mull (extracted HA) to prevent the transmission of foot and mouth disease in pigs (Schultz, 1965). Lotosh, 1991, mentioned the humate as a pharmacy that raises resistance against non-specific diseases. This fact was confirmed by using such models as atoxic anemia, toxic hepatitis, peptic ulcer and hypercholesterolemia.

Liver effects: In an experimental model with partially hepatectomised rats, long-term application of HA resulted in the stimulation of omithine decarboxylase, an increase in spermidine and histamine as well as DNA and RNA levels, and in overall liver mass (Maslinski *et al.,* 1993). It is also clear that the humate plays a role in the liver function and protects somewhat from disease and/or disturbances (Lotosh, 1991).

Odour reduction: Texas A and M University System researchers have discovered that using humate decreases volatile ammonia in animal waste by 64%, reduces odour, and improves the nitrogen to phosphorus ratio in the waste (Parker *et al.*, 2001). Scientists are developing rations formulated to enhance manure characteristics while maintaining the performance of animal (Greene and Cole, 2000; Mosley, 1996).

Residue in food material

In residue studies swine orally received a mixture of Humocarb and concentrated HA (ratio 16:1) at a dose level of 500 and 2000 mg kg^{-1} bw day^{-1} for 30 days and sheep orally received 1000 to 2000 mg kg^{-1} bw day^{-1}. At the end of the treatment periods no HA could be detected by a photometric method (limit of detection: 10 to 50 µg ml^{-1}) in blood plasma and muscle, liver and kidney (CVMP, 1999). The results obtained by different researchers (Lange *et al.*, 1996; Kühnert *et al.*, 1989) indicated that HA 1500 is toxicologically not harmful after oral administration. Taking into account the pharmacokinetic data, residues of the substance in animal tissues can be ruled out with high significance. They also found that after oral administration of 500 mg HA 1500 kg^{-1} bw, the half life period was 1.5 hours and maximum plasma concentration was 3 µg ml^{-1}.

Conclusion: There is no doubt that HA has many beneficial effect like antibacterial, antiviral and anti inflammatory in animals, improves immune system, stress management and reduce odour in faeces. It also has positive effect on liver functioning. Ultimately reduces mortality and increases growth in poultry. But the level of benefits is now questionable for ruminants because due to its antimicrobial affect may cause depression of protein synthesis by reducing rumen microorganism. Supported literature those indicates the HA as growth promoter in ruminants seems weak in this aspects and facing question. It's affect as goitrogenic substance in rat is rejected in case of poultry by recent findings. So, in relation to growth promoter, using routinely is not so positive but where health risk is higher might be reflect beneficial due to protection of diseases. It is also difficult to compare the actual effects of HA preparations due to different sources and preparations as well as because rearing of animal in various region of the world differing the climate.

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