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# Dose Titration, Tolerance and Compatibility of Some Feed Additives in Broiler

Dissertation
Submitted in fulfilment of the requirements
for the degree Ph.D. in Agricultural Sciences

to the

**Faculty Agricultural Sciences**

presented by

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Dhaka, Bangladesh

**2005**

This thesis was accepted as a doctoral dissertation in fulfilment of the requirements for the degree Ph.D. in Agricultural Sciences by the Faculty Agricultural Sciences at University of Hohenheim, on 20th July, 2005.

Date of oral examination: 20th July, 2005

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*Dedicated to my daughter*

*Fatin Anzum Khan (Tuntal)*

## *List of Abbreviations*

| Abbreviations | Meaning |
|---|---|
| AA | Amino acids |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| CA | Citric acid |
| CHO | Carbohydrate |
| CP | Crude protein |
| CF | Crude fat |
| CK | Creatine kinase |
| DMI | Dry matter intake |
| DM | Dry matter |
| EE | Ether extract |
| FCE | Feed conversion efficiency |
| FI | Feed intake |
| FA | Fumaric acid |
| FCR | Feed conversion ratio |
| GE | Gross energy |
| GI | Gastro-intestinal |
| GIT | Gastro-intestinal tract |
| γ-GT | Gamma-glutamyl transferase |
| HPLC | High performance liquid chromatography |
| HA | Humic acid (s) |
| LDH | Lactate dehydrogenase |
| LWG | Live weight gain |
| ME | Metabolizable energy |
| MIC | Minimum inhibitory concentration |
| MRL | Maximum residue limit |
| NVFA | Non volatile fatty acid |
| N | Nitrogen |
| NFE | Nitrogen-free-extract |
| SCFA | Short chain fatty acid |
| TGAC | Technical grade active constituent |
| TCA | Tricarboxilic acid cycle |
| VFA | Volatile fatty acid |

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# 1. Introduction

Farm animals of modern breed have a considerable genetic potential to produce high amount of meat, milk and eggs. Quality and safety of these food products depends upon other factors like proper herd management, quality of feed and feeding regimes. For feed quality feed additives play an important role to sustain optimum growth and most economical feed conversion, and to prevent and control diseases. Optimal dosing of feed additives and their compatibility with others and veterinary therapeutics deserve specific attention to maintain their beneficial effects in different aspects of the present farming conditions.

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) by the use of antibiotics is increasingly considered as a hazard, therefore the approval of antimicrobial growth promoters will be phased out by EU legislation by the end of 2005. Many alternatives are discussed: probiotics (microorganisms), prebiotics (fermentable fibre substances), herbal compounds and organic acids. Also humic acids, approved as veterinary drug at EU level, could be considered as such an alternative. Most of these substances exert their effects by influencing gastrointestintal flora and digestion processes. As for all feed additives approved, the alternatives have to be efficacious and safe for the target animal, the consumer, the user and their environment. Compatibility of feed additives with others (and feedstuffs and carriers) and with commonly used therapeutic agents is one aspect of safety for the target animal.

It was the aim the work presented to examine the efficacy and some aspects of safety of two compounds, an organic acid and humic acids, both approved at EU statutory level but for other purposes, as growth promoters for chickens for fattening, and to investigate compatibility of an ionophore coccidiostat with a veterinary antibiotic, worldwide used in the treatment of mycoplasmosis in poultry.

As organic acids fumaric acid has been selected for their study. Fumaric acid (FA) is approved at EU-level as a preservative to protect cereals and other feedstuffs against microbial decomposition but without minimum and maximum level for feed inclusion. It shows enough antimicrobial action (Alp *et al.,* 1999; Radecki *et al.,* 1987) to preserve the feed against bacterial and fungal spoilage, but simultaneously reduces undesirable bacteria in the gastrointestinal tract (e.g. *E. coli*) and ultimately improves growth rate of different animal species (Bolduan, 1987; Eidelsburger and Kirchgessner, 1994; Falkowski and Aherne, 1983; Patten and Waldroup, 1988) at an extent comparable to antimicrobial growth promoters. FA is involved in the production of energy (in the form of ATP) by playing an active role in the tricarboxilic acid (TCA) cycle. But, some pathological effects were observed when an ester of fumaric acid (FA) was used in human therapy of psoriasis even severe nephrotoxic effects were described (Hohenegger *et al.,* 1989). Considering these aspects the first experiment was designed as dose titration study to investigate if and to what extent the chicken for fattening responds to certain dietary levels of FA under today's production conditions (feed and

breed has remarkably changed during the last decades) and if an acceptable margin of safety between a potentially beneficial dietary concentration and the lowest intolerated inclusion level could be established.

Aim and design of the second experiment with humic acids on broilers corresponds to the first experiment. Humic acids (HA), a class of compounds resulting from decomposition of organic matter, particularly plants, are natural constituents of drinking water, soil and lignite. HA inhibit bacterial and fungal growth, thus decrease levels of mycotoxins in feed (Riede *et al.,* 1991; HuminTech, 2004). HA have positive impact on health. Beneficial effects of HA are described concerning stress management (Enviromate, 2002), immune system (HuminTech, 2004; SCAN, 1999 and Enviromate, 2002), anti-inflamatory activity (Yang *et al.,* 1996; Kühnert *et al.,* 1982), antiviral properties (Huck, *et al.*, 1991; Laub 1998a; Schultz, 1965) as well as prevention of intestinal diseases, mainly diarrhoea in humans and animals. HA improved protein digestion and trace element utilization (Kreutz and Schlikekewey, 1992, Yang *et al.,* 1996, Seffner *et al.,* 1995 and Huang *et al.,* 1994) in animals. There are also reports that routine use of HA in the feed has a positive influence on growth of broilers (Bailey *et al.,* 1996; Parks, 1998; Shermer *et al.,* 1998; Eren *et al.,* 2000; Kocabağli *et al.,* 2002; Ceylan and Ciftci 2002; Humintech, 2004). However, also contradictory findings in piglets are described (Schuhmacher and Gropp, 2000).

The third experiment was designed to study the compatibility of the coccidiostat semduramicin with tiamulin. The effect on performance and health of broilers fed semduramicin (AVIAX®) continously with co-administration of tiamulin (tiamulin hydrogen fumarate) for 5 days should be examined. The experiment should be completed by a study on carcass quality characteristics after a 5 days withdrawal period of semduramicin.

Coccidiosis, a debilitating protozoal (*Eimeria spp.*) infection in poultry, is prevented by the wide spread use of coccidiostats as feed additives. According to the today's field experience – as long as effective vaccines are not available - an economical poultry production is not possible without the use of anticoccidial agents. They are added to the feed from day one until the end of production, mostly followed by a withdrawal period (for consumer protection) of 3 to 5 days. Among ionophore coccidiostats semduramicin, a monocarboxilic acid polyether ionophore produced by fermentation of a selected strain of *Actinomadura roseorufa* (Glazer *et al.,* 1992), has a broad spectrum of anticoccidial activity against *Eimeria spp*. at dietary inclusion levels ranging from 20 to 30 ppm (Ricketts *et al.*, 1992). But optimum dose level for maximum weight gain and control of coccidial lesions in broiler chicks was confirmed to be 25 ppm (McKenzie *et al.,* 1993).

Tiamulin, a semisynthetic derivative of Pleuromutilin, is effectively used in treatment of enzootic pneumonia, dysentery and arthritis in pigs (Stipkovits *et al.,* 1977), airsacculities, respiratory and intestinal infection in chicken and pigs caused by *Mycoplasma spp.* (Laber and Schütze, 1975). Infected animals become often more susceptible to different viral infections such as PRRS (Porcine Reproductive and Respiratory Syndrome) Virus swine influenza (Thacker *et al.,* 1999) and bacterial pathogens as well as cilia-associated respiratory bacillus (Ciprian *et al.,* 1988; Caruso and Ross, 1990; Andrada *et al.,* 2002), which cause secondary infection. This will lead to

reduced growth, impaired feed conversion, more runts, increased rate of morbidity (more emergency slaughters) and mortality.

But tiamulin is not compatible with most of ionophore coccidiostats like monensin, salinomycin and narasin (Goff *et al.,* 1980; Weisman *et al.,* 1980; Fink, 1981; Horrox 1980; Frigg *et al.,* 1983; Laczay *et al.,* 1987, 1989a, 1989b). As a consequence, poultry when suffering from mycoplasmosis could not be treated with the most effective antibiotic if feed contains these ionophores. Under practical conditions considering the size of poultry operations a sudden exchange of feed is not feasible. It is therefore of outstanding importance to know if there are any ionophores, which may be tolerated by chickens under the simultaneous use of tiamulin. Recently the tiamulin producing company Novartis stated that tiamulin is compatible with the ionophore anticoccidials lasalocid and semduramicin (http://www.ah.novartis.com/products/en/_fab/tiamutin45wsg.shtml) but without giving references. The findings in literatures are not unanimous, some agree (Ricketts *et al.,* 1992; Comben, 1984) to this statement, others disagree (SCAN, 2002; FEEDAP, 2004) because the area of study was limited due to application of tiamulin to only a few days and lack of clinical and pathological examination.

# 2. Review of Literature

## 2.1 **Fumaric Acid in Animal Agriculture**

The use of organic acids is well known in pig production since 1970 (Young *et al.,* 1970) because of their growth promoting (Bolduan, 1987; Kirchgessner and Roth, 1976; Giesting and Easter, 1985) and health protecting (Alp *et al.,* 1999) properties.

### 2.1.1 **Availability and Chemistry**

Fumaric acid was isolated by Winkler in 1832 from the plant Fumitory (*Fumaria officinalis L*) and called 'Acidic boletique' because firstly detected in the fungus *Boletus pseudoignarius* (Rudy, 1967). FA is also found in poppy plants (*papaveraceae)* in higher amounts. Often FA is called 'free FA' in order to distinguish it from its salts and esters. It is an organic dicarboxylic acid having acid groups on both ends of the molecule with formula $C_4H_4O_4$ and molecular weight 116.07 (Figure 1). The acid is soluble in water and its chemical identity is not modified when dissolved.

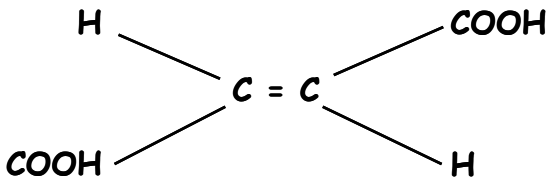


*Figure 1.* ***Fumaric acid (Trans form)***

### 2.1.2 **Metabolic Role of Fumaric Acid in the Organism**

Fumaric acid occurs naturally in the metabolism. It plays a role in the tricarboxylic acid (TCA) cycle and in the carry over of the amino-N from aspertate.

Succinate (acetyl-CoA and oxaloacetate are synthetised to citrate, which is by multiple steps- convertated to succinate) is oxidized to fumarate. Succinate also enters as succinyl-CoA the TCA-cycle as a conversion product of the glucogenic amino acids methionine, isoleucine and valine. The glucogenic amino acids phenylalanine and tyrosine enter the TCA cycle at the stage of fumarate.

Fumarate is further converted to malate (by saturation of the double bond). Oxydation of malate produces oxaloacetate. By transamination oxaloacetate is in a reversible equilibirium with aspartate.

Malate is also important outside the TCA cycle (i) as a shuttle of reducing equivalent (NADH) from cytoplasm into mitochondria, (ii) it can be converted in the cytoplasm to pyruvate (NADPH source by malic enzyme) and (iii) as a precursor of cytoplasmic synthesis of fatty acids.

Aspartate is used for protein synthesis and as a precaursor of the pyrimidine bases (the carbon skeleton is used for uracil, thymine and cytosine) and the purine bases (adenosine and guanine contain the $N_1$ from amino-N-aspartate and another amino group from aspartate on the $C_6$ of adenosine). Fumarate results from the carry over of the aspartate-amino-N via succinate.

By the same kind of reaction fumarate also results from the urea cycle in the (periportal) hepatocytes. Citrulline and aspartate form argino-succinate, which is in turn converted to arginine and fumarate. This reaction is reversible. Aspartate can again be resynthetised from fumarate via malate and oxaloacetate.

Fumarate also results from the degradation of the amino acid tyrosine (hydroxyphenylalanine). Tyrosine serves as a precursor of several syntheses (i) melanin, a pigment in skin and hair, (ii) dopamine and the catecholamines (hormones of the peripheral glands), (iii) thyroid hormones and (iv) probably ubiquinone.

Metabolic importance of FA in animal body was also identified up to a level of 3 % in many trial conducted with pigs (Table 1) but higher levels yet not studied. Recently, trial conducted with broiler also indicated its importance in energy and protein metabolism (Imangulov *et al.,* 1994). The activity of different enzyme like aspertate transferase, alanine transferase and succinate dehydrogenase was increased with the addition of FA to the diets of rat (Tschierschwitz *et al.,* 1982), suggesting this compound as a modifier of intermediary metabolism of protein and energy. Janssen, 1986, calculated the ME content of pure FA based on ATP-forming capacity is 11.35 MJ $kg^{-1}$. Günther, 1979, gives the ME of FA with 11.5 MJ/$kg^{-1}$. Another report indicated that in general organic acids have a good net energy value (higher than 10 MJ $kg^{-1}$) and are completely metabolizable as energy source comparable to glucose (JEFO, 2004). According to Blank *et al.*, 1999, FA, as a readily available energy source, may have a local

tropic affect on the mucosa in the small intestine and lead to faster recovery of the gastrointestinal epithelial cells after weaning.

*Table 1.* ***Metabolic importance of fumaric acid in animal trial***

| Diets | Animal | Level (%feed) | Criterion studied | Results | Reference |
|---|---|---|---|---|---|
| Pig starter | Piglets | 1.0-2.5 | Metabolic Importance | (+ve) | Buntenkötter, 1978 |
| Pig starter | Starter pig | 2.0-3.0 | Energy and CHO utilization | (+ve) | Giesting *et al.,* 1991 |
| Rat diet | Growing rat | 3.0 | Intermediary metabolism | (+ve) | Grassmann and Klasna, 1986 |
| Broiler starter | Broiler | 1.0-2.5 | Energy Metabolism | (+ve) | Imangulov *et al.,* 1994 |
| Complete ration | Monogastric animal | 1.0 | Peptide hydrolase activity | (+ve) | Voinova *et al.,* 1987 |

(+ve): positive

### 2.1.3 Fumaric Acid Improves Nutritive Value in Animal

Most of the scientists (Blank *et al.,* 1999, Kirchgessner and Roth, 1980 and 1988) found the nutrient digestibility and nitrogen balance as positively influenced in piglets due to use of FA as feed additive. Other scientists compared FA with citric acid (Pallauf and Walz, 1988, Zaghini *et al.,* 1986) in pigs and rabbits. They found the FA superior in improving the nutritive value at a level of 1.5 % (Table 2).

But, Falkowski and Aherne, 1984, found no significant improvement in apparent digestibility of DM or N when FA was added to a pig diet. Geisting and Easter, 1991, also reported insignificant effects on digestibility and gain applying 2 % dietary FA. Again, these conclusions are contradictory to the findings of Kirchgessner and Roth, 1980, which reported significant improvements of DM and N digestibility in weaning piglets. Supplementation of FA to starter diets during the first 3 to 4 weeks after weaning increased ileal digestibilities of GE, CP, and AAs (Blank *et al.,* 1999) in piglets.

Giesting *et al.,* 1991, found that the addition of sodium bicarbonate limiting diet acidification (pH reduction) improved the performance due to FA supplementation. The addition of FA would lead to a more favourable (acidic) milieu for pepsin activity in the stomach because Pepsin I has an optimal activity at pH 2, pepsin II at pH 3.5. Their activities decline above pH 3.6, with no activity at pH 6.0 (Taylor, 1959). So, considering above points inclusion of FA may cause better digestibility of the nutrients.

### 2.1.4 Antimicrobial Effect of Fumaric Acid

Fumaric acid shows an antimicrobial effect as propionic, citric as well as lactic acid also against opportunistic pathogens existing in gastrointestinal tract. Table 3 gives a summary on the quantitative occurrence of different groups of bacteria in the GIT of poultry.

*Table 2.* ***Effect of fumaric acid on the nutritive value of feed in animal***

| Diets | Animal | Level (%) | Criteria studied | Results | Remarks | References |
|---|---|---|---|---|---|---|
| Pig starter | Early-weaned pigs | 1.0-3.0 | Protein, energy and amino acid digestibility | (+ve) | Up to 3-4 weeks | Blank *et al.,* 1999 |
| Pig starter | Piglets | 1.0-2.0 | Nutrient digestibility, energy and protein balance | (+ve) | – | Kirchgessner and Roth, 1980 |
| Pig starter | Early weaned piglets | 1.5/3.0 | Nutrient digestibility and N-balance | (+ve) | 3.0 (NS) | Pallauf *et al.,* 1987 |
| Compare with CA in piglet | Early weaned piglet | 1.5 | N- balance | (+ve) | Better than citric acid | Pallauf and Walz, 1988 |
| Pig ration | Pigs | 2.0 | Ileal digestibility of different protein | (NS) | All protein hat similar performance | Giesting *et al.,* 1991 |
| Comparison with CA | Starter pigs | 1.5/3.0 | Nutrient balance | (NS) | Fumaric acid was best | Radecki *et al.,* 1988 |
| Compare with CA | Piglet | 1.5 | Amino acid utilization | (NS) | – | Walz and Pallauf, 1997 |
| Compare with CA | Early weaned piglet | 1.5 | N-retension | (+ve) | _ | Pallauf and Walz, 1988 |
| Pig ration | Growing pigs | 1.5 | N –retension | (+ve) | _ | Angelova, 1984 |

(+ve): positive; NS: Not significant

*Table 3.* ***Chicken intestinal microflora in percentage (Moran, 1982)***

| Group | Bacteria | Duodenum | Jejunum | Ileum | Ceca |
|---|---|---|---|---|---|
| I | *Streptococcus* | 36.6 | 8.9 | 16.8 | 0.7 |
| | *Staphylococcus* | 0.4 | - | 0.5 | - |
| II | *Lactobacillus* | 19.0 | 33.8 | 59.0 | - |
| III | *E. coli* | 5.4 | 33.0 | 14.7 | - |
| IV | Anaerobic coccus | 1.8 | 0.9 | 0.5 | 14.2 |
| V | *Eubacterium* | 26.4 | 22.6 | 7.8 | 60.6 |
| VI | *Propionibacterium* | 0.3 | 0.4 | - | - |
| VII | *Clostridium* | 1.8 | 0.4 | - | 2.1 |
| VIII | Gemminger | 1.5 | - | - | 3.4 |
| IX | *Fusobacterium* | 3.7 | - | 0.5 | 6.2 |
| X | *Bacteroides* | - | - | - | 12.8 |
| XI | Unknown anaerobes | 3.1 | - | 0.2 | - |
| % | Facultative anaerobes | 61.4 | 75.7 | 91.0 | 0.7 |
| % | Anaerobes | 38.6 | 24.3 | 9.0 | 99.3 |

Alp *et al.*, 1999, showed in broiler that FA decreased ileal microflora without hampering growth. These findings already supported by another scientist (Radecki *et al.,* 1987) in pigs. Vogt *et al.,* 1979, analysed the ingesta from small intestine and caecum of broilers fed FA containing diets and found an insignificant reduction of the total bacterial count due to less enterococci and anaerobic spore forming organisms, especially in the small intestine.

Sutton *et al.,* 1991, added 0.3 % Na-fumarate to a control diet, but did not see any significant effect on the concentration of SCFA and the density of *Lactobacilli* or *E. coli* along the GIT. But the same authors observed a decreasing effect of 1.0 % FA on *E. coli* counts in the stomach of 8 week-old piglets, and an increasing effect on VFA in the caecum compared to a control diet. No effect on VFA concentration, lactobacilli counts along the GI-tract or on *E. coli* in the duodenum, caecum, or colon was detected.

Gedek *et al.,* 1992, demonstrated a significant decrease of lactobacilli in duodenum, jejunum, ileum, caecum and colon; of eubacteria in duodenum, jejunum and ileum; of enterococci in duodenum and jejunum; and *E. coli* in the jejumum of 10 week-old piglets fed diets containing 1.8% FA. In an experiment with broiler chickens, increasing amounts of FA (0.5, 1.0 and 2.0%) did not offer protection from caecal *Salmonella* colonization or carcass contamination following oral challenge with *Salmonella typhimurium* (Waldroup *et al.,* 1995).

Poultry diets usually have high alkalinity, because they are rich in protein and nitrogenous substances and show high amounts of minerals in the diets for layers and breeders. The use of diets characterised by such a high buffer capacity can compromise the intestine capability to maintain an acidity level that can support growth and in some cases the beneficial microflora (Table 4), whereas it prevents the development of numerous pathogenic germs (Figure 2).

*Table 4.* ***Gastro-intestinal tract content pH in broiler***

| **Organ** | **Broiler (Moran, 1982)** | **Chicken (Sturkie, 1976)** | **Piglet (Hollis, 2004)** |
|---|---|---|---|
| Crop | 6.3 (4.0-7.8) | 4.51 | -- |
| Proventriculus/ stomach | 1.8 (0.3-4.1) | 4.8 | 2.0-5.0 |
| Gizzard | 2.5 (0.4-5.4) | 2.5 | -- |
| Duodenum | 6.4 (5.2-7.6) | 5.7-6.0 | 4.0-6.0 |
| Jejunum/small intestine | 6.6 (5.5-7.7) | 5.8-5.9 | 4.0-6.0 |
| Ileum | 7.2 (5.7-8.2) | 6.3-6.4 | 6.5-7.5 |
| Caecum | 6.9 (5.7-8.4) | 5.7 | 5.5-6.5 |
| Cloaca | 7.0 (5.4-8.4) | 6.3 | - |
| Colon | - | - | 6.5-7.0 |

1.5% FA lowered ($p<0.001$) the pH of the diet from 5.6 to 4.06 (Radecki *et al.,* 1988), acidification would seem to be a plausible mode of action for FA as for other orgaanic acids. So, it is possible to state that the poultry intestinal tract acidification allows modulation of the intestinal bacterial flora in a positive and natural way, and works, at the same time, against the multiplication of that bacterial flora that besides being

harmful for the animal health can represent a problem of legal nature concerning safety of feed- and foodstuffs.

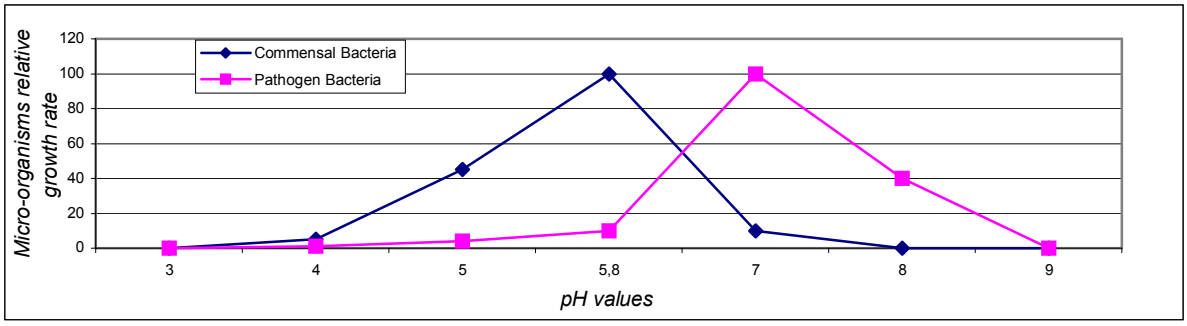


*Figure 2.* ***Effect of the intestinal pH on the growth of poultry commensal and pathogen microorganisms (Neofarma, 2004).***

The experiments studying suppression of microbial pathogens in pigs and broilers as initiated by FA were limited to rather low dietary concentrations (1.5 % and 2.0 %) (Table 5). In most cases FA treatment exhibited positive effects, and higher FA levels might have been more effective regarding this parameter but not studied.

*Table 5.* ***Use of fumaric acid as antimicrobial substance in animal feed***

| Diets | Animal | Level (%) | Criteria studied | Results | Remarks | References |
|---|---|---|---|---|---|---|
| Complete ration | Broiler | 0.3 | Ileal microflora | Reduced | Growth (NS) | Alp *et al.*, 1999 |
| Complete ration | Broiler | 2.0 | Small intestinal microflora | Reduced | _ | Vogt *et al.*, 1981 |
| Complete ration | Pigs | 1.5 | Reduction of fecal microflora | Reduced | _ | Radecki *et al.*, 1987 |
| Comparison with CA | Hybrid rabbit | 1.5 | Growth and FCR | (NS) | *E. coli* suppression | Zaghini *et al.*, 1986 |

Also it has been shown that the incorporation of organic acids into the diet of the pig reduced both gastric pH and bacterial burden along the alimentary tract (Cole *et al.*, 1968; Thomlinson and Lawrence, 1981; Scipioni *et al.*, 1978). Also Roth *et al.*, 1992, could confirm the reduction of gastric pH as a consequence of adding 1.8 % FA to the feed. A similar effect was found for 1.4 % HCl. Three hours after feeding, 1.8 % FA lowered significantly the pH value in stomach contents by 0.7 units, 1.4 % HCl by 0.3 units. I can be summarized that supplementing feed with FA lowers the gastric pH.

### 2.1.5 Growth Performance of Animal due to Fumaric Acid

Different scientists studied the effect of FA in pig starter rations up to levet of 4% on growth (Table 6). They found positive effects (Kirchgessner and Roth, 1976; Cornelius, 1988; Giesting and Easter, 1985). They also compared FA with CA and propionic acid and proved FA as better in growing pig.

In broiler rations (Table 7) the addition of 2.0 % FA was found effective in improving growth (Kirchgessner *et al.*, 1991; Patten and Waldroup, 1988).

*Table 6.* ***Use of FA as growth promoter in pigs***

| Diets | Animal | Used (%feed) | Purpose | Results | References |
|---|---|---|---|---|---|
| Pig starter | Starter ration | 2.0-3.0 | Growth | (+ve) | Giesting *et al.,* 1991 |
| Pig starter to compare with citric and propionic acid | Starter ration | 1.0-4.0 | Growth | (+ve) | Giesting and Easter, 1985 |
| Pig starter | Starter ration | 2.0 and 4.0 | Growth | (+ve) | Kirchgessner and Roth, 1976 |
| Pig starter | Starter pigs | 1.0-4.0 | Growth | (+ve) | Cornelius, 1988 |
| Pig starter diet | Starter pigs | 1.0-2.0 | Growth | (+ve) | Falkowski and Aherne, 1983 |
| Pig starter to compare with citric acid | Starter pigs | 1.0-2.0 | Growth | (+ve) | Falkowski and Aherne, 1984 |
| Pig starter to compare with propionic acid | Piglets | 0.5 and1.0 | Growth | (+ve) | Bolduan, 1987 |
| Use with other growth promoters | Pigs | 1.0-1.5 | Growth | (+ve) | Patersen and Oslage, 1982 |
| FA and CA with or without antibiotic | Starter pigs | 0,1.5, 3.0 | Growth | 1.5% (+ve) | Radecki *et al.,* 1988 |
| Pig starter | Early weaned piglet | 1.5 | Growth | NS | Henry *et al.,* 1985 |
| With or without citric acid | Early weaning piglet | 0.7 | Growth | NS | Scipioni *et al.,* 1979 |

(+ve): Positive; NS-Not significant

*Table 7.* ***Use of FA as growth promoter in avian species***

| Diets | Animal | FA (%) | Criteria studied | Results | References |
|---|---|---|---|---|---|
| Broiler starter | Broiler | 0.5-1.0 | Growth | (+ve) | Eidelsburger and Kirchgessner, 1994 |
| Levels and quality of protein varied | Broiler starter | 1.0 and 2.0 | Performance | (+ve) | Kirchgessner *et al.,* 1991 |
| To compare with Ca- formate | Broiler | 0.5-1.5 | Growth | (+ve) | Patten and Waldroup, 1988 |
| Grower ration | Growing chicken | 0.5-6.0 | Growth, heat stress | 0.5, 1.5, 3.0 (NS); 6.0 (-ve) | Silva *et al.,* 1995 |
| Starter | Broiler | 0.13-0.50 | Growth | 0.25%(+ve) | Skinner *et al.,* 1991 |
| Starter | Broiler | Upto 8.0 | Growth and FCR | FCR(+ve) | Vogt *et al.,* 1979 |
| Levels and quality of protein varied | Laying hen | 0.5 | Laying performance | NS | Kirchgessner *et al.,* 1992 |
| Layer ration | Laying hen | 0.1-0.2 | Laying and energy efficiency | (+ve) | Okolelova and Krivoruchko, 1991 |

(+ve): Positive; (-ve): Negitive; NS: Not significant

Okolelova and Krivoruchko, 1991 found positive performance in layers. When used at 2% level, FA increased the efficiency of dietary energy utilization and of its

transformation into egg weight. On the other hand Kirchgessner *et al.,* 1992, indicated insignificant effect when used only 0.5% FA in the laying hen. This insignificant effect was also supported by the data of Vogt *et al.,* 1981. The authors suggested further studies on the effect of FA on Ca-metabolism as well as shell formation.

Under the above circumstances FA at the level of 4% and 2.0% is considered effective in improving growth in pig and broiler.

A 12 years observational study in an East-Westfalen veterinary practice servicing cattle and pig farms indicated that FA, used as an acidifier in milk substitute powder and milk supplements, would lead to specific clinical signs. The pathology is characterised by acute non-inflamatory peritoneal dropsy, watery mucous swelling of the retroperitoneal tissue around the kidneys, and enlarged ochre-coloured pale kidneys with confluence of the cortical kidney lobes (Schmack, 2001). SCAN (2002) commented this publication as showing some serious shortcomings and did not follow the interpretations of the author. But SCAN (2002) could not propose an upper dietary limit due to the lack of knowledge in the absorption rate and in the nephrotoxic potential and nature of FA in calves. However, SCAN conceded that tubular dysfunctions in calves, even transient, couldn't be excluded.

### 2.1.6 **Medicinal Use of Fumaric Acid**

#### 2.1.6.1 Treatment of Psoriasis

A number of drugs as well as dietary inclusion of fish oil, vitamin D, folic acid etc. are now recognized for the treatment of psoriasis. Also, FA and its esters like FA monoethylester or FA di-ethylester has been shown in extended studies to be effective against psoriasis (Altmeyer *et al.,* 1994; Mrowietz *et al.,* 1999 and Kolbach and Nieboer, 1992).

But, due to some side effect it is important to use the FA with supervision during the treatment of psoreasis. Kidney disorders have been reported in people taking FA esters, possibly due to taking large amounts too quickly. High single oral doses (100 mg) of FA monoethylester have distinct nephrotoxic effects in the rat. Most studies humans have reported gastrointestinal upset and skin flushing as common side effects; some have also found decreased white blood cell counts with prolonged use (Kolbach and Nieboer, 1992 and Altmeyer *et al.,* 1996). But all symptoms were of transient nature.

Most studies on the antipsoriatic mode of action of dimethylfumarate (DMF) focused on its antiproliferative effects in keratinocytes. Because inflammatory skin diseases are associated with an upregulation of endothelial cell adhesion molecules and because the presence of inflammatory cell in dermis and epidermis is considered an important feature in psoriasis, Vandermeeren *et al.,* 1997 concluded that the inhibitory effect on cytokine-induced endothelial adhesion molecule expression might represent another target of dimethylfumarate in psoriasis.

The fumarate esters especially the dimethlyfumarate by its metabolite monomethylfumarate are effective in immunomodulation on psoriatric patients, but obviously not in healthy humans. There is no evidence that FA shows a comparable immunomodulating effect.

#### 2.1.6.2 Effect on Carcinogenesis

FA was examined for its effect on hepatocarcinogenesis in rats fed 3-methyl-4'-(dimethylamino) azobenzene for 50 days; subsequently they were then given a diet containing 1 % FA and drinking water containing 0.025 % FA for 51 weeks. The administration of FA effectively suppressed the development of hepatocellular carcinoma, hyperplastic nodules, and hyperplastic areas in the livers of rats (Kuroda *et al.,* 1983). The effect on carcinogenesis was also examined in a group of mice fed thioacetamide at a level of 1 % in a basal diet. FA inhibited carcinogenesis due to thioacetamide markedly; no hepatic carcinomas were found in any of the 15 animals (Akao and Kuroda, 1990). The administration of FA suppressed also the potassium 1-methyl-7-[2-(5-nitro-2-furyl)vinyl]- 4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxylate induced stomach and lung carcinogenesis (Kuroda *et al.,* 1982).

#### 2.1.6.3 Against fungal infection

Male mice were innoculated into the tail veins with yeast cells of *Candida albicans* and treated with daily intraperitoneal injections of FA at the dose of 40 mg $kg^{-1}$ bw $d^{-1}$. The results indicated that the administration of FA was effective in prolonging the survival of animals and prevented one-fifth of the treated animals from dying of candidiasis (Akao and Kuroda, 1991).

#### 2.1.6.4 Miscellaneous

Fumaric acid might have further indications (e.g. in organ transplantation) implications with regard to safety issues (e.g. immune monitoring) (Lehmann *et al.,* 2002). It was also demonstrated that the administration of organic acids including fumaric acid could be helpful in controlling scours in neonatal kids (Kritas, 2002).

### 2.1.7 **Carcass Quality of Broiler Fed Fumaric Acid**

Infection of *Campylobacter spp.* at farm level (Shanker *et al.,* 1982; Jacobs-Reitsma *et al.,* 1995) exhibited gastroenteritis and caused human health hazard. Also water plays an important role as a vehicle in the horizontal transmission route in broiler farms (Pearson *et al.,* 1993). Chaveerach *et al.,* 2002, demonstrated that adding FA or other organic acids to the water system might reduce cross-infection of *Campylobacter spp.* and helps in improving the carcass safety for human consumption.

Denli *et al.,* 2003, and Izat *et al.,* 1988, showed that beside feed conversion dietary organic acids could improve also carcass quality of broiler chicks. Vogt *et al.,* 1979 found a linear correlation between the decline in the grilling loss at the carcass and FA supplementation for 1 to 4%. Skinner *et al.,* 1991, indicated that dietary FA had no adverse effects on dressing percentage.

### 2.1.7 **Conclusions**

The use of FA in animal feed might exert several positive influences on animal production because (i) it has antibacterial as well as anti-fungal properties in processed feed as well as in the GIT of treated animals, (ii) in its long term use since middle of last century it showed evidence for better performance of avian species and pigs at dietary levels of 2-4 %. FA is obviously also effective in human therapy (as esters

against psoriasis) and revealed potential further indications (neonatal diarrhoea) and is subject for more speculative indications (carcinogenesis, organ transplantation). Side effects after oral intake are described in human medicine. The only report in animals (calves) is not reliable. So, no information on tolerance, margin of safety and potential side effects of overdosing is available for food producing animals, especially for poultry.

## 2.2 Humic Acid Substances in 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.

### 2.2.1 **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 utilisation 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 different humic substances have been identified.

#### 2.2.1.1 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 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.

#### 2.2.1.2 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.

2.2.1.3 Fulvic Acids

Fulvic acids are soluble under all pH conditions. Fulvic acids 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.

2.2.1.4 Phenolic Acids

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

## 2.2.2 **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 away. 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 as shown below (Figure 3). 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.

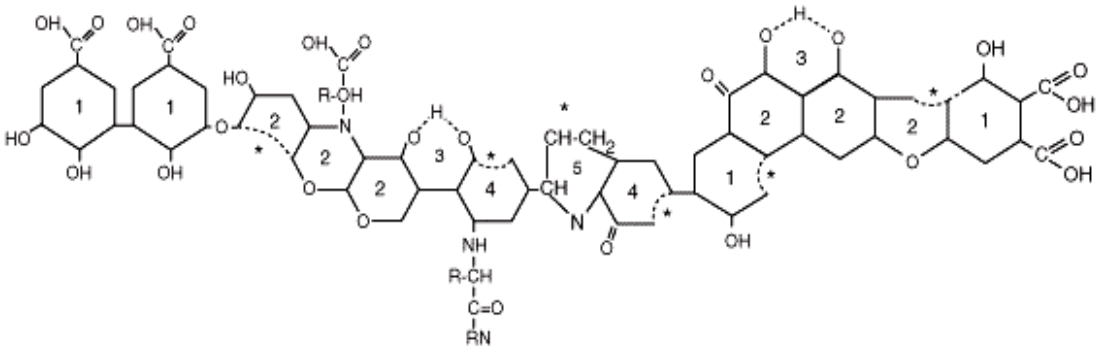

*Figure 3. Oxidized HA Molecule*

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 here. Sodium humates are preferable for animals as sodium is an important inorganic electrolyte for animal.

## 2.2.3 **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 (groups a-d) in that sample of humate was 83-94 %. The activity had been confirmed by bio-assay that had connected those bioactive organic chemicals to the effect of 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 humic acid material contained (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 Humic acid. The HA composition varies between the humic acid preparations of different companies but also between the different sources (soil), they were extracted from.

### 2.2.4 **Humic Acids as Feed Additive**

HAs are not approved as feed additive, but as veterinary drug at EU level.

#### 2.2.4.1 Improves Performance by Increasing Nutritive Value of the Feed

In recent years, it has been observed that humates included in feed and water of poultry promote growth (Bailey *et al.,* 1996; Parks, 1998; Shermer *et al.,* 1998; Eren *et al.,* 2000). Kocabağli *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 on broiler performance. In another 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 $kg^{-1}$. 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. Incorporation of humates in the feed of chickens has reduced unspecific deaths by 3 to 5% (Stepchenko *et al.,* 1991).

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 broschure 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 and calcium and trace element utilisation.

Another test had shown that the use of HA as animal feed supplement 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 in dairy cows, while problems with scours greatly decreased (Livestock R. Us, 2003). On the whole, HA should increase the animal's resistance against stress factors such as heat.

It was observed that calves born from cows that have been fed humates, had a 13.4% increase in weight within four months, when compared to the control. The bull-calves that had been fed with humates, had an increase of 21.2 %, compared to the 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 beta-globulin level increased by 32 %. It was established that the use of humates in broilers feed activated the synthetic phase of albumineous exchange. As a result, there was a 10 % increase in mass growth, 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.

#### 2.2.4.2 Animal Performance compared to Antibiotic Growth Promoter

Replacing antibiotics 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 (Humintech, 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.

### 2.2.5 **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 humic acid 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 mycotoxin, which should lead to improved gut health (Humintech, 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, humic acids also help to prevent excessive loss of water via the intestine (HuminTech, 2004). Humic acids 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.

#### 2.2.5.1 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 groups (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.

#### 2.2.5.2 Mineral Transfer

The HA acts 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. Just as fulvic acid carries life-sustaining minerals to the body, it 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.

#### 2.2.5.3 Stress Management

Literature reports that humates block or 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 feed rations while humate animals leisurely graze (Enviromate, 2002).

#### 2.2.5.4 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.

#### 2.2.5.6 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 γ-globulin fraction). Pukhova *et al.,* 1987, found that sodium humate increases the lifespan of mongrel rats exposed to lethal doses of cobalt radiation.

#### 2.2.5.7 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).

#### 2.2.5.8 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 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, humic acids 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.

#### 2.2.5.9 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).

#### 2.2.5.10 Odour Reduction

Texas A&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 animal performance (Greene and Cole, 2000; Mosley 1996).

### 2.2.6 **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 $\mu g$ $ml^{-1}$) 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 risk less 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 $\mu g$ $ml^{-1}$.

### 2.2.7 **Conclusion**

In conclusion 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 and developing immunity. Most of the literature found is from companies but scientific articles are rather limited. The reviewed articles reflect that a limited research has been conducted with chicken and specially commercial broiler, there are also strong limitations in the knowledge of HA uses as feed additive for growth.

It is really difficult to compare the actual effects of HA preparations due to different sources and preparations as well as because raising of animal in different region of the world varies as climatic conditions.

## 2.3 Coadministration of Semduramicin and Tiamulin in Animal Production

Tiamulin and semduramicin are commonly used in poultry production as an antibiotic and an anticoccidial substance. Nowadays these two drugs are facing the question regarding the compatibility of their simultaneous use in animal production.

### 2.3.1 Semduramicin is an Anticoccidial Substance

Semduramicin is coccidiostat approved at EU level for chickens for fattening at a dose range of 20 to 25 mg $kg^{-1}$ complete feedingstuff with a withdrawal time of 5 days.

Semduramicin sodium is a monocarboxilic acid polyether ionophore derived initially from a naturally occurring related ionophore, UK-58, 852, which is produced by direct fermentation of selected strain of *Actinomadura roseorufa* Huang sp. ATCC 39647 Rev (Tynan *et al.,* 1990). The chemical formulas are $C_{45}H_{76}O_{16}$ (free acid) and $C_{45}H_{75}O_{16}Na$ (sodium salt) and the molecular weight 894.5 as sodium salt. Structure is given in figure 4.

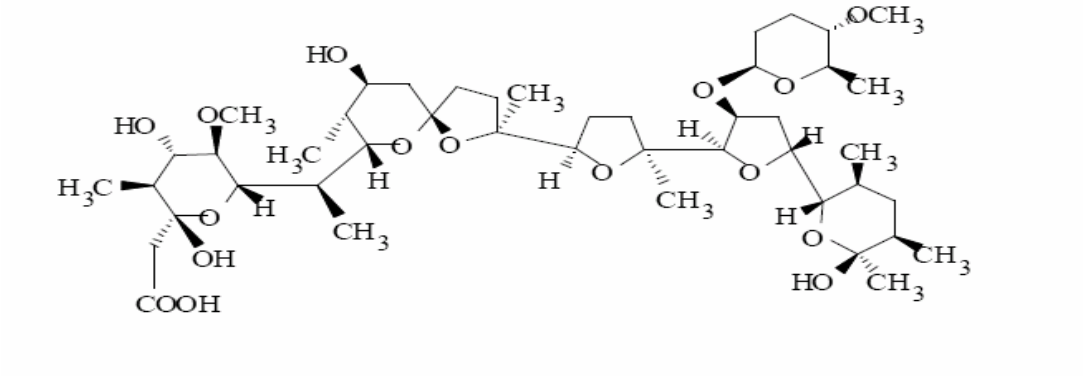


*Figure 4.* ***Chemical Structure of Semduramicin***

#### 2.3.1.1 Physico-chemical Characteristics of Semduramicin

Semduramicin sodium is available as white/off white. Its odour is as faint 'fermentation'. It is soluble in water and not corrosive during handling. It is stable for at least 2 years when stored under at or below $30^0C$ in multi walled bags.

#### 2.3.1.2 Efficacy Study of Semduramicin

It was concluded by SCAN, 2002 that the efficacy of semduramicin in terms of prevention of coccidiosis is demonstrated at the levels of 25ppm, under experimental and field conditions. At the level of 20 mg $kg^{-1}$ feed, although favourable results have been obtained, but the efficacy was not always significantly demonstrated. There is no evidence of development of resistance, using *Eimeria tenella* as test strain.

Semduramicin (25 mg $kg^{-1}$ feed) was at least as efficacious than salinomycin (60-66 mg $kg^{-1}$ feed) in controlling *Eimeria tenella, E. acervulina* in infected chickens, both anticoccidials exerted their maximum effect on weight gain and feed:gain ratio. Semduramicin also reduced *E. tenella* and *E. acervulina* lesions and coccidiosis mortality

(Conway *et al.,* 1993). Conway *et al.*, 1995 could demonstrate the effectiveness of semduramicin against *E. maxima*.

Semduramicin (26 mg $kg^{-1}$ feed) also effectively controlled mixed and monospecific infections of *Eimeria acervulina*, *Eimeria mivati/Eimeria mitis, Eimeria brunetti, Eimeria maxima, Eimeria necatrix* and *Eimeria tenella* from field isolates as seen by reduction of mortality, lesions, and weight gain depression that occurred in unmedicated, infected controls. I possesses broad spectrum anticoccidial activity (Logan *et al.,* 1993). Similar type of result obtained by McKenzie *et al.,* 1993. Semduramicin demonstrated the same activity whether produced by semisynthesis or by direct fermentation (Ricketts *et al.,* 1992). A well balanced diet with high levels of protein and methionine supports the effects of semduramicin in regard to performance and carcass parameters (Pesti *et al.,* 1999a, 1999b).

Finally, 25 mg semduramicin $kg^{-1}$ feed was determined to be optimal against *Eimeria spp*. in broiler chicks based on improved lesion control compared to 20 mg $kg^{-1}$ and improved weight gain compared to 30 mg $kg^{-1}$ (McKenzie *et al.,* 1993).

The use level is well tolerated by broilers, however with small margin of safety. But breeder hens respond to use levels with a decrease in cumulative egg production and percentage shell, an increase in early embryonic mortality. These adverse effects became evident after 1 week of exposure (Brake *et al.,* 2001).

#### 2.3.1.3 Semduramicin on the Performance of Broiler

According to McDougald *et al.,* 1996, continuous use of semduramicin (25 mg $kg^{-1}$ feed) resulted in better performance and improved lesion control in comparison with salinomicin and monensin in broiler. Shank pigmentation was improved by other three ionophores, but the improvement was superior in semduramicin groups to all other treatments.

Semduramicin ionophore was added to corn and soybean meal-based broiler diets at the recommended level, 25 mg $kg^{-1}$ feed for 0, 34, 39, or 42 day for 49 days trial results in withdrawal times of 0, 7, 10 and 15 days. Significant differences among experiments were observed but no significant differences due to semduramicin were noted in body weight or feed intake. Feeding semduramicin with a 10- or 15-day withdrawal period resulted in an improvement in feed conversion of about 0.04 units (Pesti *et al.,* 2002).

#### 2.3.1.4. Conclusion

Semduramicin at a level of 25 mg $kg^{-1}$ broiler feed is effective against commonly occurring coccidia without hampering growth with a small margin of safety. 30 mg $kg^{-1}$ seems to show a somewhat better, insignificant anticoccidial efficacy (lesion reduction) but affects feed intake and body weight gain.

The interference between semduramycin and tiamulin was not sufficiently studied. Also no data on carcass characteristics as influenced by semduramicin is available.

### 2.3.2 **Tiamulin is a Semisynthetic Antibiotic**

Tiamulin hydrogen fumarate (14-deoxy-14 [(2-diethylaminoethyl)-mercaptoacetoxy] mutilin hydrogen fumarate), is a semisynthetic derivative of the diterpene antibiotic pleuromutilin (Egger and Reinshagen, 1974) with chemical formula $C_{32}H_{51}NO_8S$ and molecular weight of 609.8. The structure in given in figure 5. Its pH varies from 3.1 to 4.1.

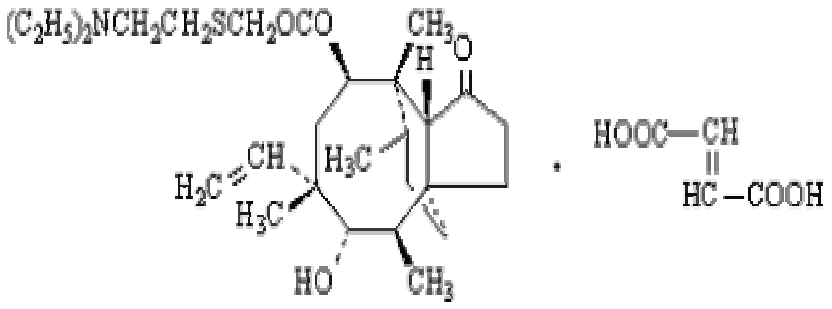


*Figure 5. Tiamulin hydrogen fumarate*

Tiamulin is *in vitro* active against 1) Mycoplasmas: *M. hyopneumoniae, M. hyorhinis, M. hyosynoviae, M. gallisepticum, M. meleagridis* 2) Spirochaetes: *Serpulina hyodysenteriae, S. innocens, S. pilosicoli, S. suis, Leptospira spp* 3) Gram-positive bacteria: *Staphylococci, Streptococci, Clostridia, Corynebacterium, Erysipelothrix, Listeria* 4) Gram-negative bacteria: *Pasteurella, Klebsiella, Haemophilus, Fusobacterium, Campylobacter, Bacteroides spp.* (Werner *et al.,* 1978, Messier *et al.,* 1990).

Poulsen *et al.,* 2001, report that tiamulin interacts with the rRNA in the peptidyl transferase slot on the ribosomes in which they prevent the correct positioning of the CCA-ends of tRNA for peptide transferase of microorganism.

#### 2.3.2.1 *In vitro* Susceptibility of Mycoplasma strains against Tiamulin

One of the predominant indications for the use of tiamulin is mycoplasma related diseases in poultry and pigs.

Valks and Burch, 2002, published recently MIC values on poultry mycoplasma strains (*Mycoplasma gallisepticum* (MG), *M. synoviae* (MS), *M. meleagridis* (MM) and *M. iowae* (MI)) over a long period of time (1975-1989, Table 8; 1990-2000, Table 9). The results indicate that those strains were and are still todays highly susceptible to tiamulin.

*Table 8.* ***Antimicrobial sensitivity ranges of various antimicrobials (µg ml$^{-1}$) against MG, MS, MM and MI, isolated between 1975 and 1989, (Valks and Burch, 2002)***

| Antimicrobial | MG (175)* | MS (53)* | MM (17) | MI (25) |
|---|---|---|---|---|
| Tiamulin | 0.0039-0.78 | 0.031-1.0 | 0.03-1.0 | 0.015-10 |
| Tylosin | 0.01-75 | 0.015-75 | 0.015-3.0 | 0.05-64 |
| Oxytetracycline | 0.12-10 | 0.06-0.08 | 0.3-5.0 | 1-3 |
| Lincomycin | 0.4-64 | 0.31-6.0 | 0.5-5.0 | 3-64 |
| Enrofloxacin | 0.0039-0.78 | 0.1-1.0 | 0.015-1.0 | 0.1-1.0 |

()Number of isolates: *Turkey and chicken isolates

*MG: M. gallisepticum; MS: M synoviae; MM: M. meleagridis; MI: M. iowae*

*Table 9.* ***Antimicrobial sensitivity ranges of various antimicrobials (µg ml$^{-1}$) against MG, MS, MM and MI, isolated between 1990 and 2000, (Valks and Burch, 2002)***

| Antimicrobial | MG (66)* | MS (52)* | MM (11) | MI (86) |
|---|---|---|---|---|
| Tiamulin | 0.006-0.39 | 0.006-0.5 | 0.025-3.13 | 0.006-0.125 |
| Tylosin | 0.006-400 | 0.006-50 | 0.78-50 | 0.05-100 |
| Oxytetracycline | 0.05-200 | 0.025-100 | 0.05-25 | 0.025-100 |
| Lincomycin | 0.125-6.25 | 0.05-1.56 | 0.05-25 | 0.05-100 |
| Enrofloxacin | 0.0125-2.0 | 0.025-1.56 | 0.1-3.13 | 0.005-1.0 |

[0]Number of isolates: *Turkey and chicken isolates

*MG: M. gallisepticum; MS: M synoviae; MM: M. meleagridis; MI: M. iowae*

The above results are supported by findings Jordan *et al.,* 1998; Ziv, 1980; Stipkovits and Kempf, 1996, and Hannan *et al.,* 1997. Mycoplasma strains isolated from pigs showed comparable MICs (Kobayashi *et al.,* 1996; Aarestrup and Friis, 1998). Also bovine Mycoplasma strains (*Mycoplasma hyosynoviae*, *M. hyopneumoniae*, *M. dispar* and *M. bovis*) are highly sensitive to tiamulin (Friis and Szancer, 1994).

2.3.2.2 Microbial Resistance to Tiamulin

According to Valks and Burch, 2002 in *Mycoplasma gallinarum* has shown no resistance development to tiamulin over the last 25 years. These findings supports by other scientists (Drews *et al.,* 1975; Stipkovits and Burch, 1993) that tiamulin is a low inducer of resistance in mycoplasma.

Bøsling *et al.,* 2003 indicated that tiamulin targets the 50S subunit of the bacterial ribosome and interacts at the peptidyl transferase centre. From studies with tiamlunin resistant *E. coli* the authors concluded that the L3 mutation, which points into the peptidyl transferase cleft, causes tiamulin resistance by alteration of the drug-binding site.

2.3.2.3 *In vivo* Effectiveness of Tiamulin

*In vivo* effectiveness of tiamulin against mycoplasma strains was evaluated in laboratory tests and confirmed later in avian species and pigs (Laber and Schütze, 1975; Stipkovits *et al.,* 1977; Baughan *et al.,* 1978). From these studies the recommended tiamulin concentration in feed for treatment of mycoplasmosis in chickens and was 0.025 % and 0.0125 % for prophylaxis.

2.3.2.4 Effect of Tiamulin on the Performance of the Animal

A piglet diet containing 100 mg tiamulin kg$^{-1}$ feed against mycoplasmal pneumonia caused *Mycoplasma hyopneumoniae* during reduced economic losses (Stipkovits *et al.,* 2003). Tiamulin improved daily gain and feed conversion over those of nonmedicated controls. The responses of both daily gain and feed efficiency increased linearly as dietary tiamulin levels increased (Hsu *et al.,* 1983). The same observation was made by Jordan *et al.,* 1998, for poultry infected by Mycoplasma.

Kleven, 1990, described that the *Mycoplasma gallisepticum* reduces egg production by 10-20 %, increases embryo mortality and chick mortality by 5-10 % and reduces weight gain and feed conversion by 10-20 %. The use of antimicrobial substances like tiamulin is

considered as the most economic method (Stipkovits *et al.,* 1993) of controlling these infections.

Trials conducted by Burch, 1984, showed that tiamulin at 30 mg $kg^{-1}$ feed significantly improved the weight gain (+4.7 %) and feed conversion of pigs (+4.7 %).

2.3.2.5 Interaction of Tiamulin with other Drugs in Farm Animals

Tiamulin is compatible with tetracyclines in broilers, turkeys and pigs (Burch and Stipkovits, 1993; Valks and Burch, 2002; Burch *et al.*, 1986). It is incompatible with nitrovin (Noa *et al.*, 2000), a former growth promoter in pigs and poultry.

Clinically important-often lethal-interactions between the ionophore anticoccidials and the antibiotic tiamulin are well known phenomena in chickens and turkeys (but also in pigs and rats) for more than two decades. This incompatibility is well established for monensin, salinomycin, maduramycin and narasin (Hanrahan *et al.*, 1981; Weisman *et al.*, 1983a; Umemura *et al.*, 1985; vanVleet *et al.*, 1987; Laczay *et al.,* 1989; Frigg *et al.,* 1983; Mazurkiewicz *et al.*, 1989a; Szucs *et al.*, 2000; Croubels *et al.,* 2001). Although the nature of this interaction remained unknown for many years, Meingassner *et al.* (1979) concluded from his findings (anticoccidial efficacy of ionophore at considerably lower levels than the routine use level under continous administration of tiamulin, increased monensin residue) that tiamulin reduces metabolic degradation and excretion of monensin in chickens. This conclusion is in agreement with the observation, that principally the same toxic symptoms (loss of appetite, locomotor disturbances, ataxia, neurotoxic symptoms) were seen after administration of monensin alone or in combination with tiamulin (Hanrahan *et al.*, 1981; Umemura *et al.*, 1985; vanVleet *et al.*, 1987; Mazurkiewicz *et al.*, 1989a; Szucs *et al.*, 2000).

The clinical symptoms (after feeding maduramycin, lasalocid, monensin, narasin or salinomycin at use levels together with tiamulin) were asociated to marked disturbances in the transport of ions, i.e. sodium, potassium, calcium, magnesium, iron, zinc, copper, between myocytes and intercellular space (Mazurkiewicz *et al.,* 1989b). Sakar *et al.,* 1991a/b demonstrated that tiamulin caused increased enzymatic levels (CK, ALD, LDH, AST, ALT and MDM) in the blood serum of pigs when administred with narasin or monensin. This indicates muscle damage. But withdrawal of both drugs caused the enzymes return to the normal level after 12 days and 1 day, respectively. Histological and ultrastructural examination of muscle tissues in broilers after administration of maduramycin, lasalocid, monensin, narasin or salinomycin at use levels together with tiamulin (20 mg kg $bw^{-1}$) revealed myo- and cardiomyopathies (Madej *et al.*, 1993). The alterations originated from primary mitochondria lesions followed by ATP deficiency, oedema, degeneration and necrosis of myocytes.

The interaction is dose dependent (Meingassner *et al.*, 1979; Lehel *et al.*, 1995; Lehel and Laczay, 1995; Weisman *et al.*, 1980; 1983, Stipkovits *et al.*, 1992). Stipkovits *et al.*, 1992, could show in laboratory and field experiments that 20-30 mg tiamulin $kg^{-1}$ feed and 60 mg salinomycin $kg^{-1}$ sustain maximum growth of broilers and are therefore compatibile at these levels.

Antioxidants obviously reduce the severity of toxic symptoms in swine and chicken (vanVleet *et al.*, 1987; Laczay *et al.*, 1994; Lehel *et al.*, 1995).

Later, further toxic interactions of other drugs with polyethers (mainly monensin) became known. Studies by Frigg *et al.*, 1983, indicate that sulphonamides increase the toxicity of monensin. Also the co-administration of chloramphenicol (Broz and Frigg, 1987), erythromycin, oleandomycin and furazolidone with monensin gave similar results (see review by Anadón and Martinez-Larrañaga, 1990; Anadón and Reeve-Johnson, 1999).

Recent data indicate that the interaction of polyethers with tiamulin and macrolide antibiotics involves their influence on the microsomal cytochrome P-450 isoenzymes, which play an important role in the oxidative and reductive metabolism of numerous endogenous and exogenous compounds. Several classes of xenobiotics are known for their interaction with cytochrome P-450 through the formation of stable metabolic intermediate complexes. After being metabolized these compounds complex the iron (II) present in the heme group of P-450, which is than enzymatically inactive and unable to bind carbon monoxide. An important group of drugs forming these metabolic intermediate complexes is the macrolide antibiotics (Larrey *et al.*, 1983; Watkins *et al.*, 1986). Tiamulin is a potent inhibitor and inducer of cytochrome P-450, via the formation of an metabolic intermediate complex. Certain N-demethylation and hydroxylation processes were strongly inhibited by tiamulin (Witkamp *et al.*, 1994). The interaction with cytochrome P-450 appears to be selective on enzymes of the P-450 3A subfamily, although some interactions with P-450-1A1 may occur (Witkamp *et al.*, 1995, 1996). Monensin itself does not exert significant effects on microsomal liver enzymes (Szucs *et al.*, 2000). However, P-450 3A plays an important role in the oxidative metabolism of monensin (Nebbia *et al.*, 1999). Compounds capable of binding or inhibiting these isoenzymes could therefore be expected to give rise to toxic interactions with the ionophore(s).

As the relative toxicities of the ionophores (within and across species) vary considerably (Oehme and Pickrell, 1999), the polyethers show remarkable differences concerning the severity of toxic interactions.

In 1984, it was shown that lasalocid sodium might be the only ionophore that could be exposed concomitantly with tiamulin without adverse effects (Comben, 1984). Sakar *et al.,* 1992 demonstrated that the activity of AST and CK was within the normal range after the continuous administration of tiamulin and lasalocid. Lodge *et al.,* 1988, confirmed also the compatibility of lasalocid (125 mg $kg^{-1}$ feed) with tiamulin (125 mg $L^{-1}$ water) in turkeys.

Moreover a laboratory study indicated that semduramicin at 20 to 30 ppm (the routine use level) was well tolerated when coadministered with tiamulin (250 mg $kg^{-1}$ feed) (Ricketts *et al.,* 1992). But this result obviously disagree with data reviewed by SCAN, 2002, indicating that the concurrent medication of birds receiving 25 mg semduramicin $kg^{-1}$ feed with tiamulin in water (250 mg $L^{-1}$) for three days resulted in a depression of weight gain and deterioration on feed efficiency. Other relevant data as haematology, clinical chemistry, gross pathology and histopathology from treated animals with this combination were not available. SCAN considered also the time of tiamulin administration as short in comparison with recent recommendations for tiamulin treatment (5 days) so that in the opinion of SCAN no proof was given for the compatibility of semduramicin and tiamulin in broilers.

# 3. Animals, Materials and Methods

All the three experimental trials were conducted in the battery house at the Faculty of Veterinary Medicine of Leipzig University, Germany (An den Tierkliniken 29, D-04103 Leipzig, Germany), which belongs to the Institute of Animal Nutrition, Nutrition Diseases and Dietetics (Gustav-Kühn-Strasse 8, D- 04159 Leipzig).

## 3.1 **Animals**

Newly hatched Lohmann-Hybrid male/female broiler chicks (Lohmann Meat) were purchased from a commercial poultry breeder (Geflügelhof Möckern, Möckern, Leipzig, Germany) and transported to the prepared broiler house at the same day. Birds were vaccinated against 'Gumburo' and 'Infectious Bursal' disease in the hatchery using spray method. At day 8 of the trials all chicks were vaccinated (Nobilis ND Hitchner, Intervet) against Newcastle disease (Virus family Paramyxoviridae) via water treatment.

## 3.2 **Experimental Designs**

### 3.2.1 Experiment 1, Fumaric Acid

The potential growth promotion efficacy of fumaric acid in chickens for fattening should be evaluated. Experiment 1 was designed as dose titration and tolerance study.

A number of 576 male day old broiler chicks were distributed to 72 cages with 8 birds each (Table 10). Six types of rations containing different amounts of FA were offered to the birds (each rations for 12 cages). The distribution of the replicate cages of each feeding groups were according to randomised block design (RBD) where the horizontal position of the cages were considered as a block (Appendix 1).

*Table 10.* ***Layout of Experiment 1***

| **Code**<br>***Fumaric acid (%)*** | **(1)**<br>***0*** | **(2)**<br>***1.25*** | **(3)**<br>***2.5*** | **(4)**<br>***3.75*** | **(5)**<br>***5.0*** | **(6)**<br>***7.5*** |
|---|---|---|---|---|---|---|
| Replicates | 12 | 12 | 12 | 12 | 12 | 12 |
| Birds/replicate | 8 | 8 | 8 | 8 | 8 | 8 |
| Chicks/treatment | 96 | 96 | 96 | 96 | 96 | 96 |

### 3.2.2 Experiment 2, Humic Acid

Experiment 2 was designed as dose titration study. The results should allow an assessment of the growth promoting potential of HA as feed additive in broiler diets.

A number of 480 birds were distributed in 60 cages with 8 birds each (Table. 11). Six types of rations (each ration for 10 cages) containing different amounts of HA were offered to the birds. The distribution of the replicate cages of each treatment was according to RBD where the horizontal position of the cages was considered as a block (Appendix 2).

*Table 11.* ***Layout of Experiment 2***

| **Code**<br>***Humic acid (mg kg$^{-1}$ feed)*** | **(1)**<br>***0*** | **(2)**<br>***300*** | **(3)**<br>***600*** | **(4)**<br>***1200*** | **(5)**<br>***2400*** | **(6)**<br>***4800*** |
|---|---|---|---|---|---|---|
| Replicates | 10 | 10 | 10 | 10 | 10 | 10 |
| Birds/replicate | 8 | 8 | 8 | 8 | 8 | 8 |
| Chicks/treatment | 80 | 80 | 80 | 80 | 80 | 80 |

### 3.2.3 Experiment 3, Interference Study with Semduramicin and Tiamulin

Experiment 3 was conducted in two phases a) Compatibility study followed by b) Carcass quality after withdrawal of semduramicin.

#### 3.2.3.1 Compatibility Study

In a tolerance study designed as growth trial it should be investigated whether the coccidiostat semduramicin (AVIAX®) and the simultaneous administration of semduramicin and tiamulin (tiamulin hydrogen fumarate) has an effect on the zootechnical parameters, the physiological status and health of female broilers chickens.

From 360 just hatched, 320 female broiler chicks were selected after one day of acclimatization to the environment, then individually weighed and randomly allocated (Table 12) ages to obtain nearly equal weight in each cage (421 to 436 g) and to form four treatment groups of identical weight (54.1 ± 0.3 g per bird).

The four treatment groups were randomly allocated to 10 blocks of four cages each, which were created according to tiers (2 x 2 and 2 x 3) and battery (4); every treatment group was represented in each block. The distribution of treatment groups is shown in Appendix 3.

*Table 12.* ***Layout of Experiment 3***

| **Code**<br>***Semduramicin in feed (mg kg$^{-1}$ feed)***<br>***Tiamulin in water (mg L$^{-1}$ water), day 15 to 19*** | **(1)**<br>***None***<br>***None*** | **(2)**<br>***None***<br>***250*** | **(3)**<br>***25***<br>***None*** | **(4)**<br>***25***<br>***250*** |
|---|---|---|---|---|
| Replicates | 10 | 10 | 10 | 10 |
| Birds/replicate | 8 | 8 | 8 | 8 |
| Chicks/treatment | 80 | 80 | 80 | 80 |

#### 3.2.3.2 Carcass Quality after Withdrawal of Semduramicin

Selected birds from control group (1) and semduramicin group (3) of the described experiment were transferred into floor pens, fed the control diet for 5 to 8 days (5 days are the minimum withdrawal time) and then slaughtered. 20 control birds and 20 semduramicin birds were distributed to 10 floor pens. Each treatment consisted of 5 replicates (floor pens) with 4 birds each.

## 3.3 Duration of the Trials

**Experiment** 1. The trial was conducted for a period of 26 days (from June 11 to July 7, 2003). Other activities like pathological and histopathological examinations continued for subsequent weeks.

**Experiment 2.** The trial was conducted for a period of 35 days (from September 3 to October 8, 2003). After completion of the trial pathological studies required several months.

**Experiment 3.** The interference trial was conducted for a period of 35 days (from July 23 to August 27, 2003), the withdrawal phase of semduramicin in the subsequent 8 days. Other related activities like pathological and histopathological examination, study on blood profiles; carcass characteristics as well as sensory parameters required several additional weeks.

## 3.4 Experimental Diets

The experimental diets were prepared at the Institute of Animal Nutrition, Nutrition Diseases and Dietetics, an officially recognized and registered premix and feedstuff manufacturer (Reg. No. $\alpha$ DE SN 1 00002; DE SN 1 00002), University of Leipzig, Gustav-Kühn-Straße 8, D-04159 Leipzig, Germany. Before preparing the rations the compositions were calculated to make the rations iso-nitrogenous and iso-caloric for experiments 1 and 3, they correspond to a conventional starter feed for broiler chicks.

A final premix was made from minerals, vitamin and trace element premix, DL-methionine and certain amounts of corn, soybean meal and soy bean oil. For the tets substance to be added (FA, HA, and semduramicin, respectively), a further (experimental) premix was made from cooked wheat starch, small amounts of corn and the experimental additives to achieve homogenous distribution. Afterwards, The main ingredients (e.g. soybean meal, corn, and gelatinised starch), the final premix and the experimental premix were mixed to the complete feed. For the experimental premix, the vitamin and trace element premix a batch mixer of 130 L volume was used. Final premix and complete feed were mixed in a batch mixer of 500 L volume. Finally, the diets were pelleted (2 mm diameter and about 3 mm length).

### 3.4.1 Rations for Experiment 1

The calculated rations for experiment 1 are shown in table 13 indicating the amount of different ingredients of formulated rations. The have iso-caloric diets FA was added to replace a mixture of wheat starch and cellulose. The chemical composition is given in Table 14.

### 3.4.2 Rations for Experiment 2

Humic acid was added to the experimental diets by replacing similar amounts of starch. Composition of the diets is seen in Table 15, the analytical data are given in Table 16.

*Table 13.* ***Composition of diets (%), Experiment 1***

| **Code**<br>***Fumaric acid (%)*** | **(1)**<br>***0*** | **(2)**<br>***1.25*** | **(3)**<br>***2.5*** | **(4)**<br>***3.75*** | **(5)**<br>***5.0*** | **(6)**<br>***7.5*** |
|---|---|---|---|---|---|---|
| Wheat | 36.200 | 36.200 | 36.200 | 36.200 | 36.200 | 36.200 |
| Soybean seed | 44.000 | 44.000 | 44.000 | 44.000 | 44.000 | 44.000 |
| Soybean oil | 8.000 | 8.000 | 8.000 | 8.000 | 8.000 | 8.000 |
| Wheat starch (gelatinised) | 5.800 | 4.850 | 3.900 | 2.950 | 1.950 | 0.050 |
| Cellulose | 1.750 | 1.450 | 1.150 | 0.850 | 0.600 | -- |
| Fumaric acid | -- | 1.250 | 2.500 | 3.750 | 5.000 | 7.500 |
| Monocalciumphosphate | 1.450 | 1.450 | 1.450 | 1.450 | 1.450 | 1.450 |
| Calciumcarbonate | 1.850 | 1.850 | 1.850 | 1.850 | 1.850 | 1.850 |
| Sodium chloride | 0.465 | 0.465 | 0.465 | 0.465 | 0.465 | 0.465 |
| Vitamin-premix[a] | 0.250 | 0.250 | 0.250 | 0.250 | 0.250 | 0.250 |
| Trace element premix[b] | 0.125 | 0.125 | 0.125 | 0.125 | 0.125 | 0.125 |
| DL-Methionin (99%) | 0.160 | 0.160 | 0.160 | 0.160 | 0.160 | 0.160 |
| ***Total*** | ***100.000*** | ***100.000*** | ***100.000*** | ***100.000*** | ***100.000*** | ***100.000*** |

[ab] Composition shown in Appendix 4 and 5

*Table 14.* ***Chemical Composition (g/100g air dry sample) of the Diets, Experiment 1***

| **Code**<br>***Fumaric acid (%)*** | **(1)**<br>***0*** | **(2)**<br>***1.25*** | **(3)**<br>***2.5*** | **(4)**<br>***3.75*** | **(5)**<br>***5.0*** | **(6)**<br>***7.5*** |
|---|---|---|---|---|---|---|
| Dry matter | 92.75 | 92.94 | 92.75 | 93.17 | 93.31 | 93.64 |
| Crude protein | 23.02 | 23.34 | 23.18 | 22.92 | 21.74 | 22.51 |
| Crude fibre | 5.18 | 5.26 | 4.58 | 4.46 | 4.47 | 4.21 |
| Ether extract | 11.02 | 10.99 | 11.04 | 11.36 | 12.56 | 14.14 |
| Ash | 6.87 | 7.08 | 6.89 | 7.05 | 7.19 | 6.92 |
| Nitrogen free extract | 46.66 | 46.27 | 47.06 | 47.38 | 47.35 | 45.86 |
| Organic matter | 85.88 | 85.86 | 85.86 | 86.12 | 86.12 | 86.72 |
| Starch | 30.54 | 28.27 | 30.16 | 29.22 | 29.40 | 29.97 |
| Sugar | 5.02 | 3.88 | 3.73 | 4.10 | 3.37 | 4.28 |
| *ME (MJ $kg^{-1}$ feed)*[a] | 13.10 | 12.61 | 12.90 | 12.86 | 13.03 | 13.90 |

[a] Calculated from FMV(ME in MJ $kg^{-1}$ = 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)

*Table 15.* ***Composition of diets (%), Experiment 2***

| **Code**<br>***Humic acid (mg kg$^{-1}$ feed)*** | **(1)**<br>***0*** | **(2)**<br>***300*** | **(3)**<br>***600*** | **(4)**<br>***1200*** | **(5)**<br>***2400*** | **(6)**<br>***4800*** |
|---|---|---|---|---|---|---|
| Soybean meal | 43.500 | 43.500 | 43.500 | 43.500 | 43.500 | 43.500 |
| Corn/Wheat | 41.200 | 41.200 | 41.200 | 41.200 | 41.200 | 41.200 |
| Soybean oil | 8.500 | 8.500 | 8.500 | 8.500 | 8.500 | 8.500 |
| Wheat starch (gelatinised) | 2.000 | 1.970 | 1.940 | 1.880 | 1.760 | 1.520 |
| HA/Huminfeed[a] | - | 0.030 | 0.060 | 0.120 | 0.240 | 0.480 |
| Calcium propionate | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| Monocalcium phosphate | 1.450 | 1.450 | 1.450 | 1.450 | 1.450 | 1.450 |
| Calcium carbonate | 1.350 | 1.350 | 1.350 | 1.350 | 1.350 | 1.350 |
| Sodium chloride | 0.465 | 0.465 | 0.465 | 0.465 | 0.465 | 0.465 |
| Vitamin premix[b] | 0.250 | 0.250 | 0.250 | 0.250 | 0.250 | 0.250 |
| Trace element premix[c] | 0.125 | 0.125 | 0.125 | 0.125 | 0.125 | 0.125 |
| DL-methionine | 0.160 | 0.160 | 0.160 | 0.160 | 0.160 | 0.160 |
| ***Total*** | ***100.000*** | ***100.000*** | ***100.000*** | ***100.000*** | ***100.000*** | ***100.000*** |

[a] Certified composition of HA/HuminFeed produced by Humintech GmbH, Heerdter Landstr. 189/D,D-40549 Düsseldorf, Germany: Water 14,50%; Ash 26,00 % DM; Humic acids 74,00 % DM; Sodium($Na_2O$) 12,00 % DM

[bc] Composition shown in Appendix 4 and 5

*Table 16.* ***Chemical Composition (g/100g air dry sample) of the Diets, Experiment 2***

| **Code**<br>***Humic acid (mg kg$^{-1}$ feed)*** | **(1)**<br>***0*** | **(2)**<br>***300*** | **(3)**<br>***600*** | **(4)**<br>***1200*** | **(5)**<br>***2400*** | **(6)**<br>***4800*** |
|---|---|---|---|---|---|---|
| Dry matter | 94.52 | 93.74 | 94.04 | 93.91 | 93.42 | 93.06 |
| Crude protein | 26.20 | 26,16 | 26.00 | 25.70 | 25.06 | 23.47 |
| Crude fibre | 4.52 | 4.42 | 4.30 | 4.30 | 4.47 | 4.30 |
| Ether extract | 11.25 | 11.30 | 11.40 | 11.26 | 11.62 | 11.25 |
| Ash | 6.94 | 6.75 | 6.92 | 6.86 | 7.00 | 7.42 |
| Nitrogen free extract | 45.61 | 45.11 | 45.42 | 45.79 | 45.27 | 46.62 |
| Organic matter | 87.58 | 86.99 | 87.12 | 87.05 | 86.42 | 85.64 |
| Starch | 25.65 | 25.41 | 25.02 | 24.85 | 28.02 | 26.86 |
| Sugar | 4.01 | 3.97 | 4.06 | 4.15 | 4.14 | 4.10 |
| Methionin | 0.47 | 0.45 | 0.46 | 0.46 | 0.46 | 0.46 |
| Cystine | 0.41 | 0.41 | 0.40 | 0.38 | 0.37 | 0.37 |
| *ME (MJ kg$^{-1}$ feed)*[a] | 12,73 | 12,69 | 12,65 | 12,54 | 13,09 | 12,52 |

[a] 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)

### 3.4.3 Rations for Experiment 3

Semduramicin was added to the experimental diets by replacing an equal amount of starch by AVIAX. Composition of the diets in seen in Table 17, the analytical data are given in Table 18.

*Table. 17.* ***Composition of diets (%), Experiment 3***

| **Code**<br>***Semduramicin in feed*** | **(1)*/(2)**<br>***None*** | **(3)/(4)**<br>***(25mg kg$^{-1}$)*** |
|---|---|---|
| Soybean meal | 46.000 | 46.000 |
| Corn | 39.700 | 39.700 |
| Soybean oil | 7.500 | 7.500 |
| Wheat starch (gelatinised) | 2.000 | 1.950 |
| AVIAX[a] | - | 0.050 |
| Calcium propionate | 1.000 | 1.000 |
| Monocalcium phosphate | 1.450 | 1.450 |
| Calcium carbonate | 1.350 | 1.350 |
| Sodium chloride | 0.465 | 0.465 |
| Vitamin premix[b] | 0.250 | 0.250 |
| Trace element premix[c] | 0.125 | 0.125 |
| DL-methionine | 0.160 | 0.160 |
| ***Total*** | ***100.000*** | ***100.000*** |

[a] AVIAX® is the trade mark for semduramicin sodium of Phibro Animal Health s.p.r.l., Rue de I'Institut, 87a, B-1330 Rixensart, Belgium. The current authorized dosage range in the EU and most other countries is 20 to 25 mg semduramicin per kg of complete feed (0.04 % to 0.05 % of the AVIAX® 5 % Premix).

[bc] Composition shown in Appendix 4 and 5

* Diet also used in withdrawal period

*Table 18.* ***Chemical Composition (g/100g air dry sample) of the Diets, Experiment 3***

| **Code**<br>*Semduramicin in feed* | **(1)/(2) Batch 1**<br>*None* | **(1)/(2) Batch 2**<br>*None* | **(3)/(4) Batch 1**<br>*(25mg kg$^{-1}$)* | **(3)/(4) Batch 2**<br>*(25mg kg$^{-1}$)* |
|---|---|---|---|---|
| Dry matter | 94.22 | 93.55 | 93.69 | 94.53 |
| Crude protein | 24.62 | 24.16 | 24.28 | 24.34 |
| Crude fibre | 4.85 | 4.46 | 4.73 | 4.33 |
| Ether extract | 12.04 | 12.13 | 11.60 | 12.06 |
| Ash | 7.50 | 6.90 | 7.08 | 7.13 |
| Nitrogen free extract | 45.21 | 45.90 | 46.00 | 46.67 |
| Organic matter | 86.72 | 86.65 | 86.61 | 87.4 |
| Starch | 17.37 | 23.62 | 24.60 | 25.34 |
| Sugar | 5.37 | 4.67 | 4.64 | 4.68 |
| Methionin | 0.44 | 0.43 | 0.45 | 0.45 |
| Cystine | 0.40 | 0.35 | 0.42 | 0.37 |
| *ME (MJ kg$^{-1}$ feed)*[a] | 11.55 | 12.46 | 12.46 | 12.75 |
| Semduramycin (mg kg$^{-1}$)[b] | <1 | <1 | 25.45 | 25.50 |

[a] 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)

[b] Analysed by the Institute Européen de l'Environnement de Bordeaux (I.E.E.B.), Direction Agroalimentaire, 1 Rue du Professeur Vezes, F-33300 Bordeaux, France

The tiamulin solution (Tiamulin is the trade name for tiamulin hydrogen fumarate of the Novartis Animal Health GmbH (Animal Health GmbH, Biochemiestraße 10, A-6250 Kundl/Tirol, Austria, the water soluble preparation contains 45.0% active substance). was prepared daily with fresh tap water (approximately 10°C) to obtain a concentration of 250 mg tiamulin $L^{-1}$ water. At each day of tiamulin application 8 samples of the tiamulin water solution were taken and stored at -78°C until tiamulin analysis by a validated HPLC method conducted in an accredited and registered laboratory of the Free State of Saxony (Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8.2; Gustav-Kühn-Straße 8, D-04159 Leipzig, Germany).

The analysed tiamulin concentrations in water are presented in Table 19. Generally, there exists an high correspondence between the desired and analysed concentrations, and also among the individual samples; except at day 16 in sample 2 and at day 17 in sample 7 the tiamulin content was clearly below (238 mg $L^{-1}$; -5 %) the calculated concentration, however, this should rather be attributed to a slight inaccuracy in sampling or analysis than to the preparation of the solution, because the other samples of the respective day were in the range of 250 mg $L^{-1}$. Therefore, together with the chemical analysis of the experimental diets, semduramicin and tiamulin analyses suggest, that potential group differences in the zootechnical and physiological parameters of the broiler chicks could be clearly attributed to the dietary treatment.

*Table 19.* ***Analysed tiamulin concentrations (mg $L^{-1}$ water)[1], Experiment 3***

| Day of trial | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|
| Sample 1 | 246 | 253 | 249 | 257 | 253 |
| Sample 2 | 251 | 238 | 254 | 246 | 253 |
| Sample 3 | 242 | 254 | 258 | 246 | 251 |
| Sample 4 | 242 | 243 | 237 | 249 | 260 |
| Sample 5 | 247 | 240 | 252 | 254 | 254 |
| Sample 6 | 241 | 255 | 244 | 255 | 251 |
| Sample 7 | 243 | 243 | 238 | 248 | 246 |
| Sample 8 | 255 | 241 | 257 | 250 | 251 |
| Mean | 246 | 246 | 249 | 250 | 252 |
| ±s | 5 | 7 | 8 | 4 | 4 |

*[1]Analyzed by the Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8.2, Gustav-Kühn-Straße 8, D-04159 Leipzig, Germany*

## 3.5 Housing and Management

Poultry house was well cleaned and disinfected using recommended disinfectants before starting each trial. All the cages were marked according to the distribution of different treatments in each trial.

The battery unit comprises 72 cages (steel wire) in 3 tiers. In each cage of a surface area of 0.80 $m^2$ (100 cm x 80 m x 60 cm) eight animals were housed. Right below of each cage dropping plates are arranged to collect the manure and spilled feed. In the first days of trial the floor of the cages was fully covered with three layers of absorptive paper, which was replaced every two days.

Feed was administered in high-grade steel troughs, which were located outside right in front of each cage. The feeder allows the simultaneous feed intake of 8 - 10 birds. Cup waterers were arranged alongside in the cage (4 waterer per cage). During the third week of experiment 3, water (with and without tiamulin) was offered in separate plastic waterer. Throughout the experimental period the chicks had ad libitum access to feed and water. When powdered feed residues (mash) were observed in the trough, then they were promptly exchanged with fresh pelleted feed.

Electric light (double Neon bulbs) illuminated the trial house over 24 hours. The trial house was heated on a temperature of about 34°C four days before housing the chicks. In the course of the trial temperature was gradually reduced from 34°C at start to 22°C at the end of the experiment. Temperature and ventilation were controlled automatically in a continuous way by one side in the trial house. Additionally, climate data were recorded by thermo-hygrometers located between at the window front and in cage 32 in a height of approximately 90 and 150 cm, respectively.

Initially the birds were weighed and distributed according to similar average weight (for each trial) in every cage and subsequently weighed with 7 days interval (exceptionally after 5 days interval at last week for experiment 1) at same time of the day (8.15 a.m. Dead losses and culled animals were recorded with date and approximate time of loss, number of the cage, weight of the bird and of trough including feed and likely reason for loss. In all chicks died or culled a post mortem section was conducted at the Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig (An den Tierkliniken 33, D-04103 Leipzig, Germany). If the chicks were suspected of having infectious diseases, pathologists referred them or their parts to the Landesuntersuchungsanstalt (Bahnhofstraße 58, D-04448 Wiederitzsch, Germany) for bacteriological and if necessary for virological examination. In case of mortality feed consumption of the cage was corrected by measuring feed intake until the day at which mortality occurred, and for the remaining days of the week for the reduced number of birds:

$$[\text{feed offered}_{n_i} - \text{feed refusal}_{n_i} - \text{spilled feed}_{n_{ij}} \cdot a_i] \cdot n_i +$$
$$[\text{feed offered}_{n_j} - \text{feed refusal}_{n_j} - \text{spilled feed}_{n_{ij}} \cdot a_j] \cdot n_j$$

*$n_i$ = No. of chicks until day of mortality, $n_j$ = No. of chicks after mortality occurred, $a_{i/j}$ = ratio of days until/after mortality to 7 days.*

Dead chicks were completely excluded from the calculation because weight and weight gain was calculated per bird, a correction for dead birds was therefore not necessary.

After completion of the trial subsequent work was conducted according to necessity of the research objective in different experiments as below.

### 3.5.1 Experiment 1

After finishing the trial 60 birds were selected (based on average cage weight) from 60 cages (1 bird from 10 cages each per treatment) after 26 days and killed for necropsy. During pathological examination the carcass, heart, liver and spleen were weighed. From the control group and the group with the highest FA-level smaller parts of the visceral organs were taken and preserved for further histological studies. Abnormal organs of birds of other groups were also taken.

3.5.2 Experiment 2

At the end of 35 days trial 60 chicks (based on average weight of cage) were selected from 60 cages (1 bird from 10 cages each per treatment) for necropsy. After gross necropsy the thyroid gland was removed.

3.5.3 Experiment 3

After completion of the main trial 40 birds (one bird per cage) were selected and blood samples were taken at day 36.

The birds used for blood sampling (one bird per cage; 10 birds per group) were killed for a general pathological examination; additionally, the intestine was examined for coccidia.

Also at the end of 35 days trial 40 healthy chicks (based on average weight of cage) from treatments 1 and 3 (two birds from each cage) were selected, marked and transferred into small floor pens (4 animals per pen) with concrete floor and wood shavings. The trial house with the dimension of 14.10 m x 11.45 m x 3.9 m comprises 48 pens with concrete floor; 10 of the pens were used for the extended fattening period. Each pen has a surface of 2.33 $m^2$ (2.45 m x 0.95 m x 1.00 m). Metal partitions separate pens. Every second day new shaving was added.

During the extended fattening period all birds received the pelleted control feed for at least 5 days (day 35 to 40-43; withdrawal phase). Broiler had *ad libitum* access to feed and water. Beginning with the fifth day of the extended fattening period 5 birds from each dietary group (control/withdrawal) were randomly selected, weighed and slaughtered every day at the same time until day 8 (Fig. 6). The combined weight of head

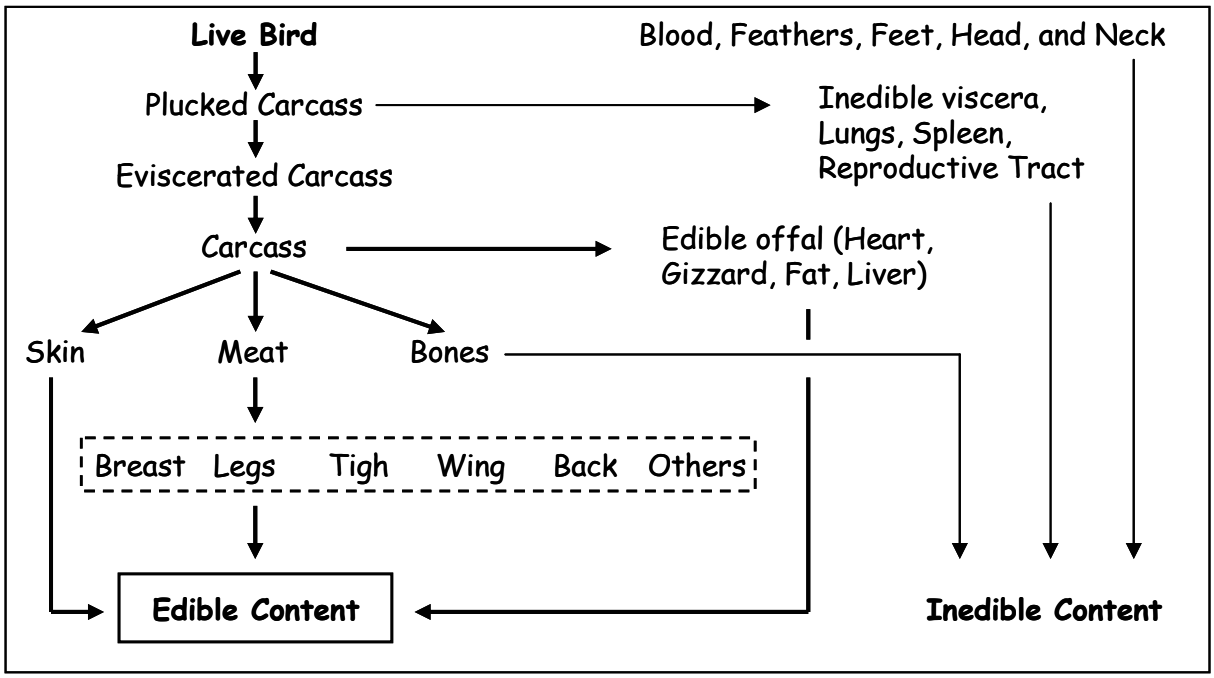


*Fig 6.* ***Slaughtering scheme of killed birds, Experiment 3***

head and neck was recorded before plucking; the weight of shanks, of whole viscera and heart, liver, muscle stomach and hot carcass was determined after wet plucking. Carcass, shanks and the giblets (heart, liver, stomach) were kept at $4^0C$ for further analyses in an accredited and registered laboratory of the Free State of Saxony (Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8.3; Gustav-Kühn-Straße 8, D-04159 Leipzig, Germany). After the overnight cooling all parts were weighed again. Then, the portions of breast (skin, small and large muscle), leg (skin, muscle, bone, fat) and wings were determined. Sensory characteristics of cooked and broiled breast and thigh muscles were evaluated by a panel consisting 3 to 4 (at the third testing day) trained persons.

## 3.6 Parameters

In all trials the zootechnical parameters live weight, live weight gain, feed intake, feed conversion efficiency, and mortality were determined.

### 3.6.1 Experiment 1

Weight of selected organs (liver, heart, and spleen), histopathology of birds from control and 7.5 % FA groups, histopathology of the evidently affected organs irrespective of all groups.

### 3.6.2 Experiment 2

One bird per pen was necropsied. Weight and size of the thyroid gland was determined.

### 3.6.3 Experiment 3

Parameters measured in blood samples were protein, albumin, glucose, uric acid, electrolytes and enzymes were determined at the Large Animal Clinic for Internal Medicine, Faculty of Veterinary Medicine, University of Leipzig (An den Tierkliniken 11, D-04103 Leipzig, Germany). The Clinic for Birds and Reptiles of the Faculty of Veterinary Medicine, University of Leipzig (An den Tierkliniken 23, D-04103 Leipzig, Germany) was responsible for the determination of haematocrit and differential blood count.

General gross necropsy was performed and histology done at liver, kidney, heart and muscle tissue (breast). Liver was examined for glycogen content (Best'carmin staining). In order to detect potential cell damages caused by the simultaneous administration of semduramicin and tiamulin the muscles and N. ischadicus of the chicks belonging to group (4) and group (1) were assessed in particular. In case of macroscopically obvious symptoms of muscle or nerve damage the affected tissues should be examined microscopically and also the birds of group (2) and (3). Pathological examinations were performed at the Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig (An den Tierkliniken 33, D-04103 Leipzig, Germany) and the tests for coccidia at the Institute of Parasitology, Faculty of Veterinary Medicine, University of Leipzig (An den Tierkliniken 33, D-04103 Leipzig, Germany).

Carcass parameters: Weight at slaughter, weight gain per pen until slaughter, dressing percentage, weight of viscera and hot/cooled dressed carcass, shanks and giblets (heart, liver, gizzard), portion of breast (skin, small and large muscle), legs and thighs (skin, muscle, bone, fat) and wings, protein and fat content of breast and legs.

Organoleptic test: Tenderness, juiciness, aroma, general impression of cooked as well as fried thigh, legs and breast.

## 3.7 Sampling

### 3.7.1 Feed

Pelleted feed was taken randomly from many (about 6) of the bags of the individual experimental diets. Mixing of all partial samples lead to a representative bulk sample of that experimental diet. A quarter of bulk samples were taken as sub sample. The sub samples were ground to make fine, ready for analysis after labelling.

### 3.7.2 Birds for necropsy/blood sampling/semduramicin withdrawal trial

After end of the each trial all birds were weighed individually, the mean value of each cage was calculated and the birds nearest to mean value were taken for necropsy/blood sampling/subsequent trial of semduramicin withdrawal phase. The birds were individually marked at a leg by plastic band.

### 3.7.3 Blood

Blood from selected birds was collected from the wing vein at the end of feeding trial of experiment 3 using syringe containing heparin as anticoagulant. The samples were labelled and preserved in the refrigerator below $4^0C$ until analysis.

### 3.7.4 Tiamulin containing water

Tiamulin containing drinking water was prepared at days 15 to 19 of experiment 3. From these preparations, 8 samples a day were taken for analysis and stored deep frozen until analyse at -78°C.

## 3.8 Chemical Analysis

Proximate components of feed were determined according to VDLUFA (2000) in the Institut für Tierernährung, Ernährungsschäden and Diätetik, and semduramicin at the Institut Européen de l'Environnement de Bordeaux (I.E.E.B.), Direction Agroalimentaire, 1 Rue du Professeur Vezes, F-33300 Bordeaux, France. Tiamulin content in water was analysed by a validated HPLC method in a LUFA-laboratory of the Free State of Saxony (Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8.3; Gustav-Kühn-Straße 8, D-04159 Leipzig, Germany). Also the proximate analysis of carcass of selected birds for experiment 3 was conducted at the mentioned LUFA-laboratory.

## 3.9 Calculation and Statistical Analysis

Data were organized for mean, standard deviation, relative values and for further analysis using computer Excel program. Organized data were statistically analysed using SPSS 11.5 for windows program SPSS, 1993 to determine the analysis of variance (ANOVA) and Tukey's B test according to Steel and Torrie (1980) for group differences. For the experiment 3 (carcass quality) few data were analysed using student-t test to know the variation between two means in control and semduramicin group.

# 4. Results

## 4.1 Fumaric Acid, Experiment 1

### 4.1.1 Growth Performance

The trial started with day old chicks having mean weight of 48 g (Table 20). After one week of the trial highest ($p<0.05$) live weight (213 g) was achieved in the 1.25 % FA group followed by 2.5, 0 (control), 3.75, 7.5 and 5.0 % FA groups with 201, 194, 193, 188 and 181 g, respectively. The effect became clear after the second week (Appendix 6) when 1.25 % FA group reached highest body weight (587 g, +7 % to control). But, growth of 5.0 and 7.5 % FA groups was lowest with 482 and 503 g ($p<0.05$ to all other groups). On the other hand the 2.5 and 3.75 % FA groups showed live weight similar (558 and 537 g for 2.5 and 3.75 % FA group, respectively) to control. This pattern of live weight as observed after 14 days continued for the third week as well as the end of trial. After 26 days 1.25 % FA group performed best (1597 g, +6 % to the control, significantly better than all other groups), 5.0 (1342 g) and 7.5 % (1378 g) FA group worse ($p<0.05$ to the other groups) and 2.5 (1532 g), 3.75 % (1485 g) and 2.5 % FA (1532 g) groups were similar to control (1506 g). When we considered the died birds to calculate final weight per cage then we found 1.25 % and 2.5 % FA group similar and 3.75 % was similar to control. In this respect highest performance was reflected also by 2.5 % FA group ($p<0.05$).

The effect of FA on the weight gain per week was already reflected at first week, where significantly higher weight gain was shown for 1.25 % FA group (165 g). Significantly lowest gain was observed in 5 % FA group (133 g). For the last 5 experimental days, only some significant differences occurred, only 1,25 % FA (461 g gain) was significantly better than 5.0 (424 g) and 7.5 % FA (411 g).

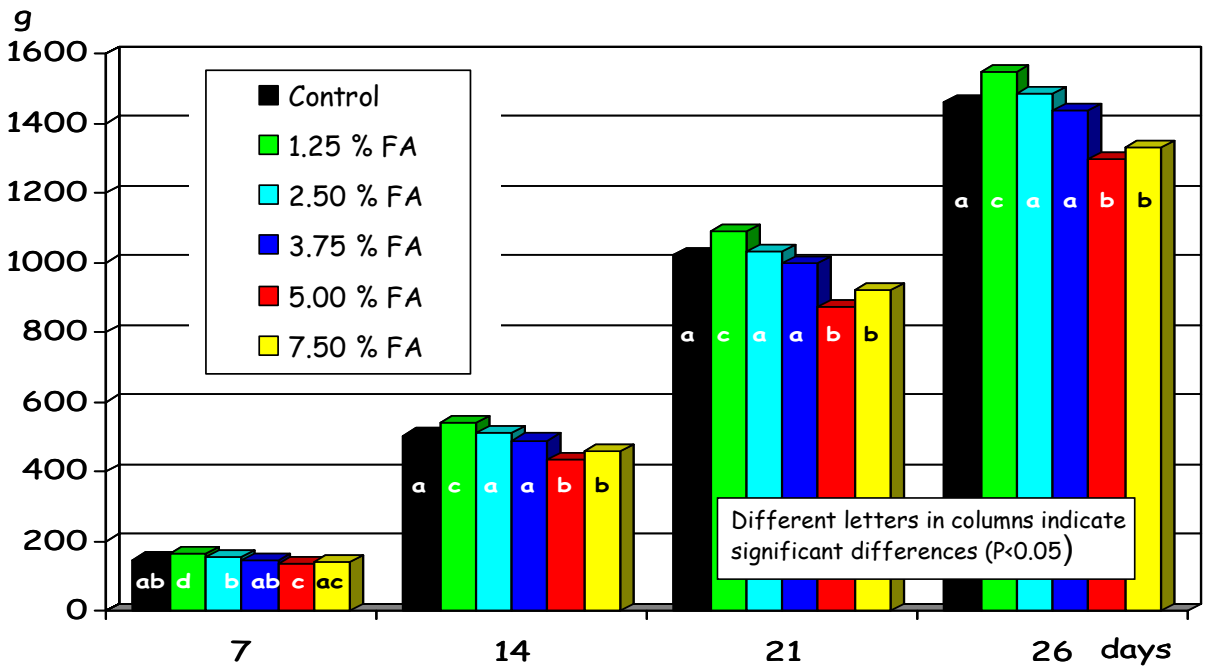


*Figure 7.* ***Fumaric acid, Experiment 1, Cumulative Live Weight Gain (g)***

Cumulative weight gain is the best indicator of growth performance (Figure 7). It appears that the 1.25 % FA group performed best and showed significant highest gain

after 21 days of age (1088 g) as well as at the end of trial (1549 g). The result also revealed that 2.5 and 3.75 % FA group gained similar to control at the age of 21 days as well as at the end of the trial. But 5.0 % and 7.5 % FA containing groups showed lowest gain (871 and 919 g, respectively) after 21 days as well as 1295 and 1331 g at the last week of the trial was lower than all other groups ($p<0.05$). It can be concluded that concerning live weight gain the addition of 1.25 % FA significantly improves weight gain, that the optimum dose is lower than 2.5 %, because higher doses up to 3.75 % remained at the control level, and that already 5.0 % FA is not tolerated by the target animal. If 1.25 % FA are considered as the highest dose, the margin of safety for FA in Chickens for fattening would be 3 (3.75/1.25).

*Table 20.* ***Growth performance (LWG and FI) and FCE (g LWG $kg^{-1}$ FI) of fumaric acid fed broiler chicks at 26 days of trial (Initial live weight: 47.6 g)***

| ***Code*** | ***(1)*** | ***(2)*** | ***(3)*** | ***(4)*** | ***(5)*** | ***(6)*** |
|---|---|---|---|---|---|---|
| ***Fumaric acid (%)*** | ***0*** | ***1.25*** | ***2.50*** | ***3.75*** | ***5.00*** | ***7.50*** |
| Final LW/bird | $1506^{a}$ | $1597^{c}$ | $1532^{a}$ | $1485^{a}$ | $1342^{b}$ | $1378^{b}$ |
| (±sd) | (83) | (49) | (48) | (48) | (65) | (49) |
| *Relative to (1)* | *100* | *106* | *102* | *99* | *89* | *92* |
| LWG/bird | $1459^{a}$ | $1549^{c}$ | $1484^{a}$ | $1438^{a}$ | $1295^{b}$ | $1331^{b}$ |
| (±sd) | (82) | (49) | (48) | (48) | (65) | (49) |
| *Relative to (1)* | *100* | *106* | *102* | *99* | *89* | *91* |
| FI/bird | $1930^{ac}$ | $2022^{c}$ | $1969^{ac}$ | $1898^{a}$ | $1754^{b}$ | $1781^{b}$ |
| (±sd) | (107) | (71) | (69) | (98) | (92) | (71) |
| *Relative to (1)* | *100* | *104* | *102* | *98* | *91* | *92* |
| FCE, | $756^{ac}$ | $767^{c}$ | $754^{ac}$ | $759^{ac}$ | $738^{b}$ | $747^{ab}$ |
| (±sd) | (10) | (9) | (16) | (21) | (17) | (16) |
| *Relative to (1)* | *100* | *102* | *100* | *101* | *98* | *99* |
| Mortality (%) | 2.1 | 3.1 | 0 | 3.1 | 4.2 | 1.0 |
| *(No. birds/treatment)* | *(2)* | *(3)* | *(0)* | *(3)* | *(4)* | *(1)* |

$^{abc}$Different superscript in the same row differ significantly ($p<0.05$)

### 4.1.2 Feed Intake

It became evident for all experimental periods that 1.25 % FA showed the highest feed intake (after one week: 172 g compared to 158 g of the control; $P<0.05$) by 1.25 % FA group at the first week of trial. The relative difference to the control group decreased from week 1 (+9%) to week 2 and 3 (+4 and 2 %, respectively). But for the last 5 days, the difference increased to significant 6 percent (Appendix 7). The feed intake of the groups with 2.5 and 3.75 % FA in feed was in all periods not significantly different from that of the control. But the 5.0 % FA group showed already after 7 days a lower feed intake (146 g, -8 %) than the control ($P<0.05$). The relative difference to the control increased during the following weeks to 12 and 13 % ($P<0.05$). Only for the last 5 days, the feed intake of this group (625 g) was similar ($P>0.05$) to the control (641 g). The lower feed intake of the 7.5 % FA group reached significance only in week 2 (-11 %) and 3 (-10 %).

The cumulative feed intake (Figure 8) at the end of the trial revealed that 1.25 and 2.5 % FA did not lead to a significantly higher feed intake than the control diet, despite

remarkable numerical differences (e.g. 2022 g for 1.25 % FA versus 1930 g of the control). But again, 5.0 and 7.5 % FA had significantly lower feed intake (-9 and -8 %, respectively, compared to the control group) than all other groups. Consistently to the conclusion drawn from growth, 5.0 % FA has to be considered as nontolerated FA dietary concentration.

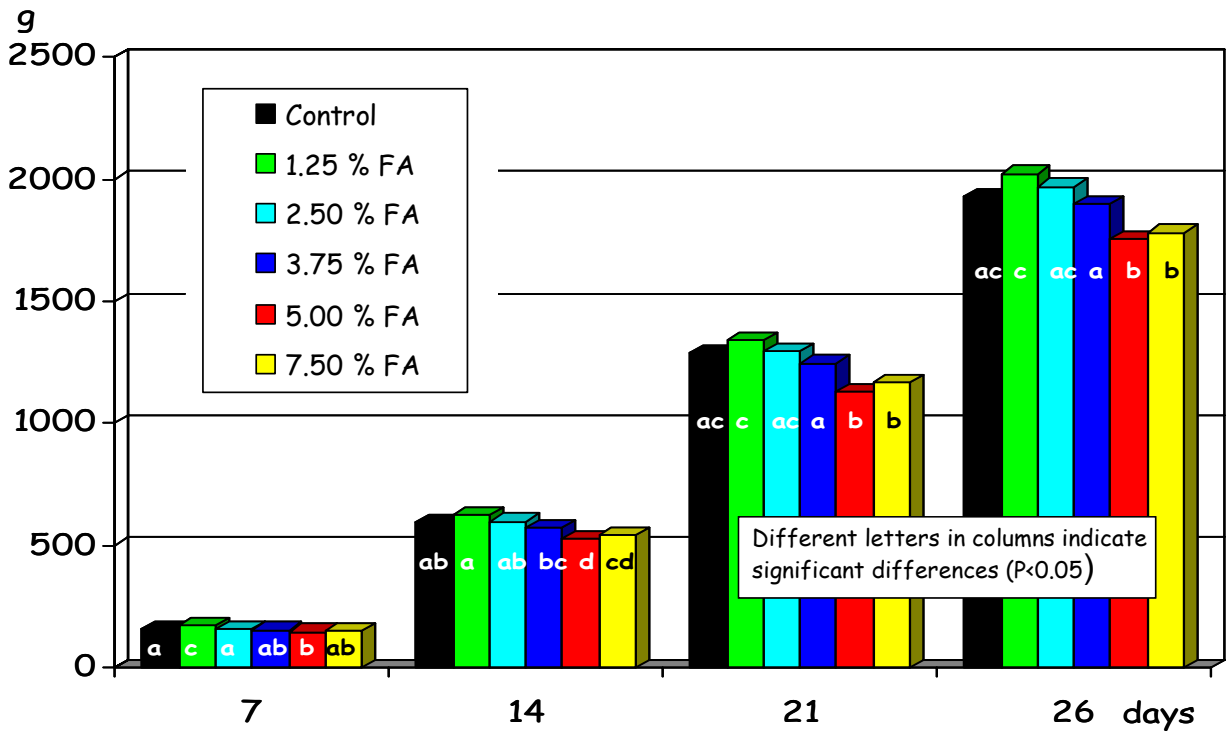


*Figure 8.* ***Fumaric acid, Experiment 1, Cumulative Feed Intake (g)***

### 4.1.3 Feed Conversion Efficiency

Values for FCE (g LWG $kg^{-1}$ feed intake) also reflect a similar trend as weight gain and feed intake (Table 8 and Appendix 8). After one week, FCE was for all groups at an similar level except the 5.0 % FA group, which was 2 % below the control value (929 g $kg^{-1}$) and significantly below the 1.25 and 2.5 % FA groups. After 14 days control, the groups with 1.25, 2.50, 3.75 and 7.5 % FA showed FCE values of comparable magnitude ($p>0.05$). It seemed that lower feed intake and lower gain resulted in approximately equal FCE values. Alone the 5.0 % FA group revealed a significantly lower FCE (820 g $kg^{-1}$) than the groups with 0, 1.25, 2.50 and 3.75 % FA (average 854 g $kg^{-1}$). After 21 days the significantly best FCE data was established by 2.50 % dietary FA, an effect, which continued until the end of the experiment (Figure 9). Also 5.0 % FA remained significantly below the 4 groups mentioned above. Moreover, the 7.5 % FA group showed a numerically low FCE value at the end of the trial (747 g $kg^{-1}$), which was not significantly different from the control (756 g $kg^{-1}$).

In conclusion also FCE data indicate 5.0% dietary FA as not tolerated by the chicken for fattening. The overall results indicate that the inclusion of 1.25% FA in the broiler diet has twofold beneficial effect stimulating growth and feed intake, both resulting in a better feed conversion. Also 2.5 and 3.75 % FA deserve attention showing similar performance like control group without any adverse effect in mentioned parameters.

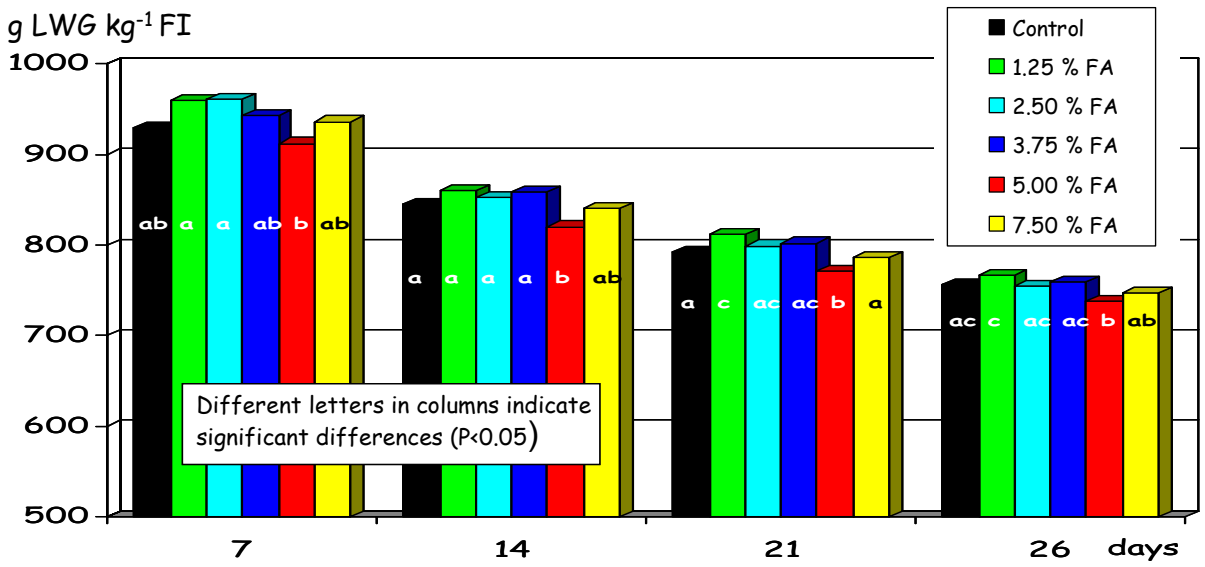


*Figure 9.* ***Fumaric acid, Experiment 1, Cumulative FCE (g LWG kg$^{-1}$ FI)***

### 4.1.4 Pathological Condition and Organ Weight

No incident of abnormalities was observed in the FA groups due to continuous inclusion of FA up to the 5% level (Table 21), which could be attributed to treatment. The 7.5 % acid-containing group showed some abnormalities in different organs (liver, kidney and "all

*Table 21.* ***Abnormalities observed in the treatment groups, experiment 1***

| **Code** | | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|---|
| Liver | Enlarged liver | - | - | 1* | 1 | - | 1# |
| | Hepatomegalie | - | 1 | - | - | - | - |
| | Bright liver | - | - | 1, 1# | 1 | 1# | - |
| Heart | Enlarged | - | 1 | - | - | - | - |
| Kidney | Radish clay in colour | - | - | - | - | 1 | 1 |
| | Grey in colour | - | - | - | - | - | 1 |
| | Radish brown marble | - | - | - | - | - | 1# |
| | Capsulated blood | - | - | 1# | - | 1# | - |
| Pancreas | Bloody | - | 1 | - | - | - | - |
| Breast Muscle | Spotted | 1 | - | 1* | - | - | - |
| All muscle | Spotted | - | - | - | - | - | 1# |
| Duodenum | Dilatation | - | - | - | - | - | 1, 1 |
| **Birds with abnormalities** | | **1** | **3** | **3** | **2** | **2** | **5** |
| **Normal** | | **9** | **7** | **7** | **8** | **8** | **5** |
| **Total birds** | | **10** | **10** | **10** | **10** | **10** | **10** |

#* Similar superscript in the same treatment indicates same bird

muscle" tissue). A mentionable effect was observed in kidneys, 3/10 birds showing some discoloration (and 2/10 in the 5.0 % FA group). Also a dilatation of duodenum was recorded in 2/10 birds of this group. Adding all abnormal findings without assessing the seriousity of the individual findings, the 7.5 % FA group counts 5/10 birds with some pathological remarks, the control group 1, and the other groups between 2 and 3.

The weight of heart and spleen showed similarity in all groups although those came from the birds of had different mean weight according to treatment at the age of 26 days. On the other hand the weight of liver showed different mean weight in different treatment groups. Despite some significance, the differences are rather considered (Table 22) accidental.

*Table 22.* ***Weight in gram of investigated birds (n=10) and their different organs (heart, liver and spleen)***

| **Code**<br>*Fumaric acid (%)* | **(1)**<br>*0* | **(2)**<br>*1.25* | **(3)**<br>*2.5* | **(4)**<br>*3.75* | **(5)**<br>*5.0* | **(6)**<br>*7.5* |
|---|---|---|---|---|---|---|
| Live weight | $1540^{a}$ | $1675^{b}$ | $1583^{a}$ | $1544^{a}$ | $1394^{c}$ | $1432^{c}$ |
| (±sd) | (100) | (48) | (75) | (61) | (72) | (104) |
| *Relative to (1)* | *100* | *109* | *103* | *100* | *91* | *93* |
| Heart | 12.2 | 12.6 | 13.1 | 12.6 | 12.5 | 12.1 |
| (±sd) | (1.32) | (1.96) | (1.79) | (1.65) | (1.90) | (1.52) |
| *Relative to (1)* | *100* | *103* | *107* | *103* | *102* | *99* |
| Liver | $45.4^{ab}$ | $48.6^{a}$ | $47.1^{a}$ | $43.7^{ab}$ | $38.7^{b}$ | $42.0^{ab}$ |
| (±sd) | (6.67) | (7.76) | (7.53) | (3.59) | (5.91) | (6.00) |
| *Relative to (1)* | *100* | *107* | *104* | *96* | *85* | *93* |
| Spleen | 2.7 | 2.7 | 2.8 | 2.9 | 2.7 | 2.6 |
| (±sd) | (0.67) | (0.67) | (0.42) | (0.32) | (0.68) | (0.52) |
| *Relative to (1)* | *100* | *100* | *104* | *107* | *100* | *96* |

$^{abc}$ Different superscripts in the same row differ significantly ($p<0.05$)

*Table 23.* ***Relative organ (heart, liver and spleen) weight (percent of body weight) of different treatment groups***

| **Code**<br>*Fumaric acid (%)* | **(1)**<br>*0* | **(2)**<br>*1.25* | **(3)**<br>*2.5* | **(4)**<br>*3.75* | **(5)**<br>*5.0* | **(6)**<br>*7.5* |
|---|---|---|---|---|---|---|
| Heart | $0.79^{ab}$ | $0.75^{b}$ | $0.83^{ab}$ | $0.82^{ab}$ | $0.90^{a}$ | $0.85^{ab}$ |
| (±sd) | (0.08) | (0.11) | (0.09) | (0.12) | (0.15) | (0.10) |
| *Relative to (1)* | *100* | *95* | *104* | *103* | *113* | *107* |
| Liver | 2.94 | 2.90 | 2.97 | 2.83 | 2.77 | 2.93 |
| (±sd) | (0.34) | (0.48) | (0.39) | (0.21) | (0.40) | (0.30) |
| *Relative to (1)* | *100* | *99* | *101* | *96* | *94* | *100* |
| Spleen | 0.18 | 0.16 | 0.18 | 0.19 | 0.19 | 0.18 |
| (±sd) | (0.04) | (0.04) | (0.03) | (0.02) | (0.05) | (0.03) |
| *Relative to (1)* | *100* | *92* | *101* | *108* | *111* | *104* |

$^{ab}$Different superscript in the same row differ significantly ($p>0.05$);

This conclusion is supported by equal (P>0.05) differences in relative liver weight. However, percentage value for the heart showed lighter heart in 1.25 % FA group, and highest value was recorded in the 5 % FA group (Table 23).

## 4.2 Humic Acid on the Performance and Health of Broiler

### 4.2.1 Growth Performance

Initially average weight of the birds was similar for all treatment groups (46.4±0.1 g) and changes were already observed at onset of experiment 2 (Table 24 and Appendix 9). Pattern of weight gain drastically changed first and second week of age by showing lower growth in the group with 4800 mg HA $kg^{-1}$. At day 7 that group had a body weight of 174 g/bird, which was lower (P<0.05) than for all other groups (193, 194, 192, 192 and 185 g for groups 1, 2, 3, 4 and 5, respectively). Similarly at day 14 this group had 483 g LW, which was again lower (P<0.05) than that of the other groups (547, 541, 543, 536 and 521g for group 1, 2, 3, 4 and 5, respectively). After second week the 4800 mg HA $kg^{-1}$ group began to compensate the weight gain depression showing after 3 weeks similar body weight as the 2400 mg $kg^{-1}$ feed group (991 and 1037 g for groups 6 and 5, respectively).

*Table 24.* ***Growth performance (LWG and FI) and FCE (gLWG/1000 g FI) of humic acid sodium salt fed broiler chicks***

| ***Code*** | ***(1)*** | ***(2)*** | ***(3)*** | ***(4)*** | ***(5)*** | ***(6)*** |
|---|---|---|---|---|---|---|
| *Humic acid (mg $kg^{-1}$ feed)* | *0* | *300* | *600* | *1200* | *2400* | *4800* |
| Final LW | 2408 | 2369 | 2335 | 2355 | 2310 | 2301 |
| (±sd) | (60) | (107) | (115) | (115) | (114) | (109) |
| *Relative to (1)* | *100* | *98* | *97* | *98* | *96* | *96* |
| Total LWG | 2361 | 2323 | 2289 | 2309 | 2263 | 2255 |
| (±sd) | (60) | (107) | (115) | (115) | (114) | (109) |
| *Relative to (1)* | *100* | *98* | *97* | *98* | *96* | *95* |
| Total FI | 3266 | 3264 | 3202 | 3256 | 3230 | 3271 |
| (±sd) | (122) | (121) | (131) | (132) | (162) | (123) |
| *Relative to (1)* | *100* | *100* | *98* | *100* | *99* | *100* |
| FCE | $723^{a}$ | $712^{ab}$ | $715^{ab}$ | $709^{ab}$ | $701^{ab}$ | $689^{b}$ |
| (±sd) | (22) | (22) | (22) | (26) | (21) | (22) |
| *Relative to (1)* | *100* | *98* | *99* | *98* | *97* | *95* |
| Mortality (%) | 8.75 | 5.00 | 1.25 | 2.50 | 1.25 | 5.00 |
| *(No of died birds/treatment)* | *(7)* | *(4)* | *(1)* | *(2)* | *(1)* | *(4)* |

$^{abc}$Different superscript in the same row differ significantly (p>0.05); [1] Considering only the live birds

At the end of week 4 group 6 showed similar body weight (1622) as the HA groups 2, 3, 4, and 5 weighing 1691, 1681, 1689 and 1652 g, respectively. But was still inferior (P<0.05) to the control (1723g). Finally. After 5 weeks all groups showed similar (P>0.05) LW as 2408, 2369, 2335, 2355, 2310, and 2301 g for groups 1, 2, 3, 4, 5, and 6, respectively.

Weekly data indicated lower (P<0.05) weight gain for the high HA group (4800 mg $kg^{-1}$) for first (129 g) and second week (309 g) as shown in Appendix 10. All other groups

reflected similar (P>0.05) weight gain during first two weeks. This type of adverse effect in high HA group tended to disappear from third week. All groups showed similar weight gains (533, 531, 533, 538, 515 and 507 g groups 1, 2, 3, 4, 5 and 6, respectively). Again similar weight gains were observed in week 4 (643, 619, 605, 614, 615 and 632 g for groups 1, 2, 3, 4, 5 and 6, respectively). In last week (5) of experiment the range of weight gains was between 679 (group 6) and 685 g (group 1) and id not show any statistically significant differences (P<0.05).

Cumulative weight gain (Figure 10) reflects similar pattern as did live weight in the corresponding weeks. Despite the tendency of the initially slower growing groups to compensate the lower gain in the following weeks, the control showed best weight gain at the end, and body weight gain was negatively correlated with the HA content of the diets (y (weight gain) = 2327.98 – 0.0181x (HA content), r= -.280*).

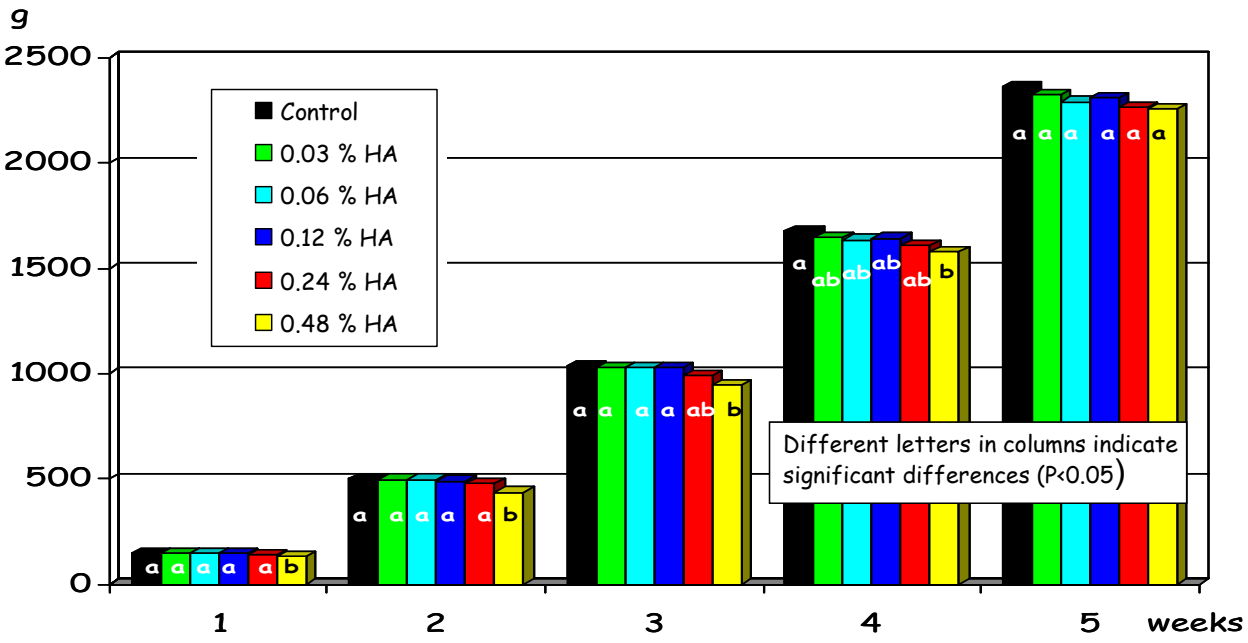


*Figure 10.* ***Humic acid, Experiment 2, Cumulative Live Weight Gain (g)***

### 4.2.2 Feed Intake

At first week of the trial the birds showed no specific pattern in feed intake with increment of HA, however the high HA group 6 revealed lowest (P<0.05) feed intake (138 g) but similar (P>0.05) to control as well as groups 3 and 4 (Appendix 11). Group 2 and 5 consumed higher amounts of feed than group 6 but similar to control, and to group 3 and 4. But the situation became stable at the second week showing similar intake as 400, 399 and 380 g for groups 4, 5 and 6, respectively, with higher HA. But feed intake of these groups was still lower than for the control and groups 2 and 3. For the following the weeks all groups showed similar values of feed consumption.

Cumulative feed consumption also specifies the adverse (P<0.05) effect of highest acid group (6) after two weeks (518 g compared to 551, 555, 553, 547 and 550 g of groups 1, 2, 3, 4 and 5, respectively). From three weeks on insignificant variations were observed in all HA groups, also compared to the control (P>0.05). Total feed consumption ranged from 3202 to 3271 g for groups 3 and 6 (P>0.05). In summary, feed consumption data

showed the same pattern as body weight gain (Table 25). Additions of HA to broiler diets depressed feed intake at the beginning of the experiment and recovered within 35 days more or less completely (Figure 11).

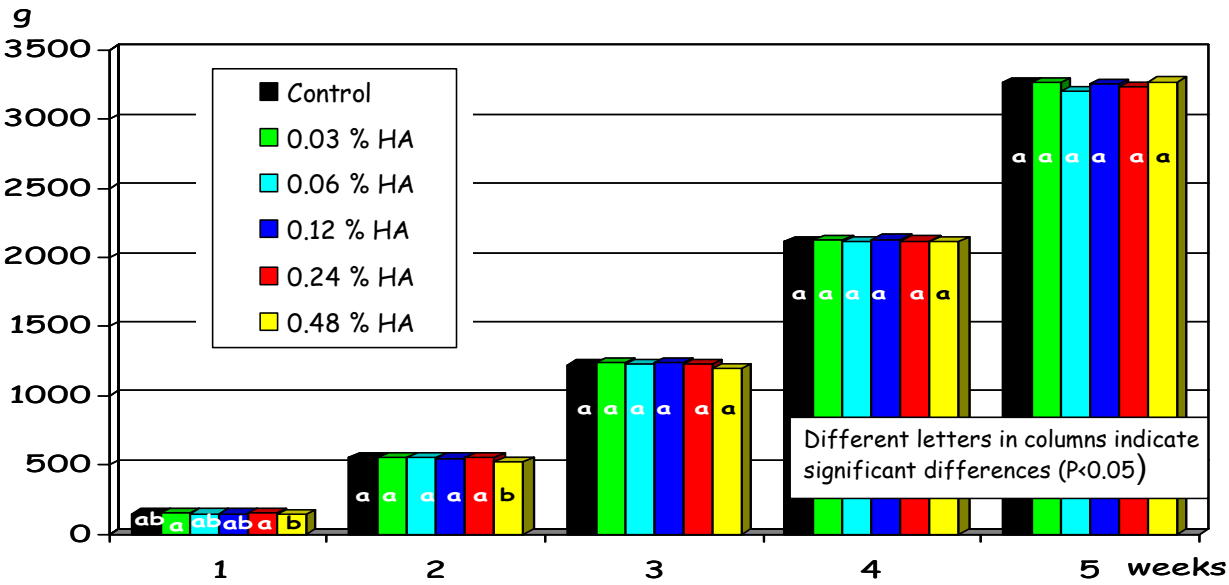


*Figure 11.* ***Humic acid, Experiment 2, Cumulative Feed Intake (g)***

### 4.2.3 Feed Conversion Efficiency

Similar feed conversion efficiency (g LWG/kg$^{-1}$ FI) was observed (Figure 12 and Appendix 12) at first week in the 3 low HA groups 2 (988), 3 (1020) and 4 (992 g kg$^{-1}$) and the control group 1 (1011 g kg$^{-1}$). These groups were better (P<0.05) than the high HA groups 5 (921) and 6 (930 g kg$^{-1}$). During second week the situation started to recover showing similar values among groups 2 (857), 3 (858), 4 (859) and 5 (842 g kg$^{-1}$), only group 6 (4800 mg HA kg$^{-1}$) performed less efficiently (813 g LWG kg$^{-1}$). At third week the same group showed similar efficiency with the 2400 mg HA kg$^{-1}$ group. Fourth and fifth weeks were stable and similarly efficient concerning conversion of feed to weight gain for all treatment groups.

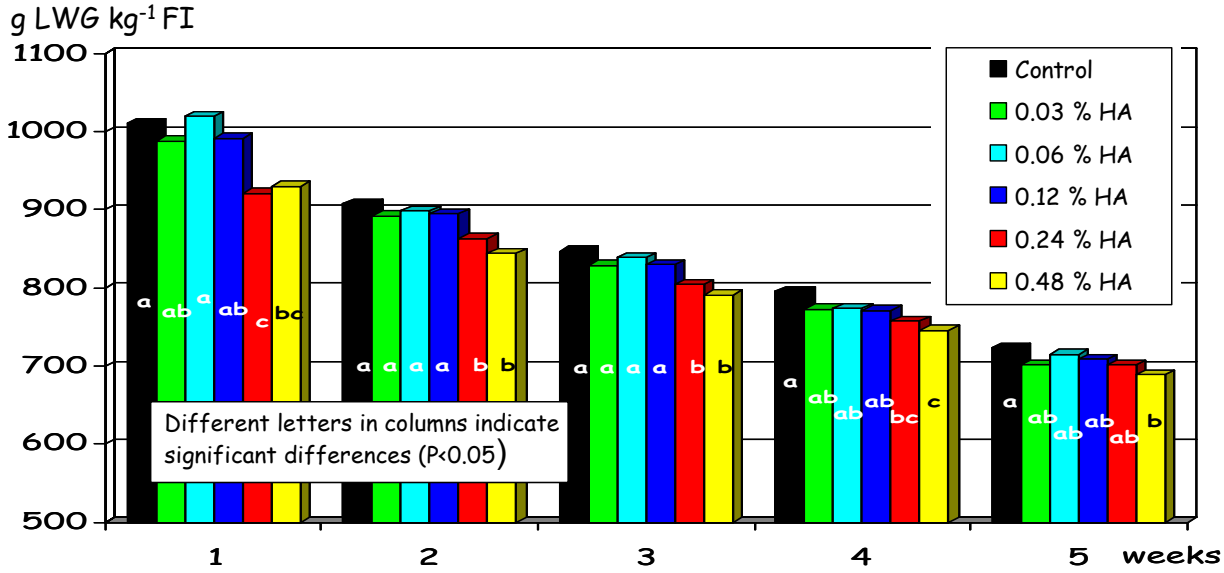


*Figure 12.* ***Humic acid, Experiment 2, Cumulative FCE (g LWG kg$^{-1}$ FI)***

The cumulative feed conversion efficiency is shown in Appendix 12 and Figure 12. There was also a tendency to show the lesser efficiency from the beginning of trial in the higher HA groups. The situation improved in subsequent weeks and finally at the end of the trial after 35 days all HA groups showed similar feed conversion efficiency except 4800 mg HA group that showed poorer efficiency than control ($P<0.05$). However, independent from any potential tendency for compensation and from weekly statistical evaluation, the control group showed best values at all times. Figure 12 conveys the impression of a gradual decline of FCE with increasing amount of humic acid in feed.

Highest mortality (8.75 %) was observed in the control group (Table 24). On the other hands the birds offered HA feed showed lower mortality rates with 5.0, 1.25, 2.50, 1.25 and 5.0 % in groups 1, 2, 3, 4 and 5, respectively. But the number of animals in the experiment was to low to base conclusions on that finding.

### 4.2.4 Pathological Condition and Weight of different Organs

The most frequent findings were liver degeneration (Table 25). In some chicks fractures and subluxatios were sporadicall observed, which are obviously of traumatic origin, which however might be attributed to the handling of the birds or to manipulations during blood sampling. Other observations had to be assessed as coincidental findings, which did not show any treatment related frequency distribution. Alterations of the thyroid gland could macroscopically not be found.

*Table 25.* ***Abnormalities observed in the treatment groups, experiment 2***

| **Code** | | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|---|
| Liver degeneration | Mild/moderate/high | 4 | 7 | 6 | 5 | 4 | 4 |
| Proventriculus and Gizard | Dilatation (Mild) | 2 | - | - | - | - | - |
| Gizard | Dialation (High) | - | - | - | 1 | - | - |
| Hydroperikard | Mild and moderate | - | 2 | - | - | 3 | - |
| | Mild | - | - | 1 | - | - | - |
| Aspiration of blood | - | 2 | 1 | - | - | - | - |
| Serofibrinous Serositis | Mild | - | 1 | - | - | - | - |
| Splenomegalie | Mild | - | 1 | 1 | 1 | - | - |
| Hypodermis inflamation | Mild | - | 1 | - | - | 1 | - |
| Decubitus (legs) | Mild | - | 1 | - | 2 | 1 | - |
| Hypodermis edema | Mild | - | 1 | - | - | 2 | - |
| Hydroankle | Mild | - | - | 1 | - | 1 | - |
| Fracture of humerus | Mild | - | - | 2 | - | 1 | - |
| Lung edema | Mild | - | - | - | 1 | 2 | - |
| Subluxatio shoulder ankle | - | - | - | - | - | 1 | - |
| **Birds with abnormalities** | | **6** | **8** | **8** | **7** | **8** | **4** |
| **Normal** | | **4** | **2** | **2** | **3** | **2** | **6** |
| **Total birds** | | **10** | **10** | **10** | **10** | **10** | **10** |

The absolute weight of liver (mean 52.3 g), spleen (mean 2.2 g) and Bursa Fabricii (4.1 g) did not differ among the treatments (Table 26). The Bursa Fabricii had been chosen as a rough indicator for potential effects of the HA on the immune system. That there are no differences in organ weights is supported by the relative organs weights, given in Table 27, showing also no differences. The maximum figure for the liver is 2.34 %, the minimum 2.19 % of bw. The corresponding range for spleen is 0.08-0.11. The Bursa Fabricii showed a remarkable wider range (2.92-4.46 % of bw), but treatment related effects can not be seen, also taking in regard the considerable standard deviation of the mean values (1.24-2.43).

*Table 26.* ***Weight (g) of investigated birds (n=10) and their different organs (liver, spleen and Bursa fabricii), experiment 2***

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|
| *Humic acid (mg kg$^{-1}$)* | *0* | *300* | *600* | *1200* | *2400* | *4800* |
| Live weight | 2376 | 2379 | 2308 | 2324 | 2310 | 2294 |
| (±sd) | (70) | (100) | (150) | (120) | (90) | (110) |
| *Relative to (1)* | *100* | *100* | *97* | *98* | *97* | *97* |
| Liver | 52.50 | 55.38 | 50.62 | 53.75 | 51.17 | 50.36 |
| (±sd) | (6.96) | (9.45) | (4.78) | (7.53) | (3.36) | (7.97) |
| *Relative to (1)* | *100* | *105* | *96* | *102* | *97* | *96* |
| Spleen | 1.99 | 2.65 | 2.27 | 2.32 | 1.97 | 2.03 |
| (±sd) | (0.54) | (0.92) | (0.88) | (0.70) | (0.68) | (0.51) |
| *Relative to (1)* | *100* | *133* | *114* | *116* | *99* | *102* |
| Bursa fabricii | 4.01 | 4.77 | 3.80 | 4.88 | 3.74 | 3.38 |
| (±sd) | (1.24) | (2.43) | (1.95) | (1.31) | (1.30) | (1.39) |
| *Relative to (1)* | *100* | *119* | *95* | *122* | *93* | *84* |

[abc]Different superscript in the same row differ significantly (p>0.05);

*Table 27.* ***Relative organ weight (liver, spleen and Bursa fabricii) in percent of body weight of the treatment groups, experiment 2***

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|
| *Humic acid (mg kg$^{-1}$)* | *0* | *300* | *600* | *1200* | *2400* | *4800* |
| Liver | 2.21 | 2.34 | 2.20 | 2.31 | 2.22 | 2.19 |
| (±sd) | (0.32) | (0.44) | (0.21) | (0.28) | (0.18) | (0.33) |
| *Relative to (1)* | *100* | *106* | *99* | *104* | *100* | *99* |
| Spleen | 0.08 | 0.11 | 0.10 | 0.10 | 0.08 | 0.09 |
| (±sd) | (0.02) | (0.04) | (0.04) | (0.03) | (0.03) | (0.02) |
| *Relative to (1)* | *100* | *132* | *116* | *118* | *111* | *105* |
| Bursa fabricii | 4.22 | 4.46 | 3.23 | 4.15 | 2.92 | 3.26 |
| (±sd) | (2.64) | (1.99) | (2.01) | (1.75) | (0.86) | (1.68) |
| *Relative to (1)* | *100* | *106* | *77* | *98* | *69* | *77* |

[abc]Different superscript in the same row differ significantly (p>0.05);

The thyroid gland was particularly examined considering the report of Seffner (1995). If HA depresses intestinal iodine uptake, a compensating increase in weight or size of

that gland could have been expected. But in fact, the criteria chosen did not reveal any treatment effect on the thyroid gland (Table 28). Especially no HA dose related effect could be seen. The maximum differences in the weight of the left and right thyroid between treatments amounted to 20 µg, which is negligible particularly in regard of the difficulties to prepare the entire thyroid from the carcass.

*Table 28.* ***Mass and size of thyroid gland in the treatment groups, experiment 2***

| **Code**<br>*Humic acid (mg kg$^{-1}$Feed)* | **(1)**<br>*0* | **(2)**<br>*300* | **(3)**<br>*600* | **(4)**<br>*1200* | **(5)**<br>*2400* | **(6)**<br>*4800* |
|---|---|---|---|---|---|---|
| Thyroid gland, left (g) | 0.11 | 0.11 | 0.13 | 0.13 | 0.12 | 0.11 |
| (±sd) | (0.03) | (0.03) | (0.03) | (0.04) | (0.04) | (0.03) |
| *Relative to (1)* | *100* | *103* | *114* | *117* | *113* | *99* |
| Thyroid gland, right (g) | 0.12 | 0.14 | 0.13 | 0.13 | 0.12 | 0.12 |
| (±sd) | (0.03) | (0.05) | (0.03) | (0.04) | (0.02) | (0.05) |
| *Relative to (1)* | *100* | *117* | *110* | *114* | *101* | *107* |
| Thyroid gland, total (g) | 0.23 | 0.25 | 0.25 | 0.26 | 0.24 | 0.23 |
| (±sd) | (0.04) | (0.07) | (0.05) | (0.07) | (0.05) | (0.07) |
| *Relative to (1)* | *100* | *110* | *112* | *115* | *107* | *103* |
| Thyroid gland, total (% bw) | 0.009 | 0.012 | 0.011 | 0.011 | 0.011 | 0.010 |
| (±sd) | (0.001) | (0.002) | (0.002) | (0.003) | (0.002) | (0.003) |
| *Relative to (1)* | *100* | *109* | *116* | *119* | *110* | *107* |
| *Thyroid, left (size in cm $^3$)** | 0.15 | 0.15 | 0.16 | 0.19 | 0.18 | 0.16 |
| (±sd) | (0.05) | (0.05) | (0.05) | (0.05) | (0.07) | (0.07) |
| *Relative to (1)* | *100* | *99* | *109* | *125* | *119* | *106* |
| *Thyroid, right (size in cm $^3$)** | 0.15 | 0.19 | 0.17 | 0.19 | 0.19 | 0.18 |
| (±sd) | (0.05) | (0.09) | (0.05) | (0.07) | (0.06) | (0.09) |
| *Relative to (1)* | *100* | *126* | *114* | *124* | *125* | *121* |

* Size is length x depth x width, overestimating volume; $^{abc}$Different superscript in the same row differ significantly ($p>0.05$);

## 4. 3 Coadministration of Semduramicin and Tiamulin in Broiler

### 4.3.1 Course of the Trial

During the experimental period of 35 days a total of 14 birds (4.4 % mortality) died or were culled. Reasons for culling were mainly "straddling" (osteochondrosis of the tibiotarsus of juvenile broiler chicks). Throughout the entire course of trial no therapeutic treatment was necessary. Table 29 informs of mortalities within the treatment groups.

Although, most of the mortalities occurred in the control group 1, a frequency distribution which could be clearly traced back to the dietary (water) treatments is not apparent, particularly because the reasons for mortality were mainly acute cardiovascular failure (myocarditis, fibrosis or degeneration of myocard) and disorders of the muscles or bones (myopathy, myositis, osteochondrosis), both frequent causes for losses in commercial broiler fattening. The relatively high mortality rate (controlled institute conditions) may be in sum also the result of unfavourable temperature conditions. Almost the entire course of trial outdoor temperatures exceeded 30°C at

*Table 29:* ***Frequency and date of mortality, experiment 3***

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| **Semduramicin** (mg $kg^{-1}$ feed) | — | — | **25** | **25** |
| **Tiamulin** (mg $L^{-1}$ water) 5 days* | — | **250** | — | **250** |
| No. of replicates (cages) | 10 | 10 | 10 | 10 |
| Chicks per replicate | 8 | 8 | 8 | 8 |
| No. of chicks at start | 80 | 80 | 80 | 80 |
| No. of died or culled (%) | 7 (8.8) | 1 (1.3) | 4 (5.0) | 2 (2.5) |
| *Relative to (1)* | *100* | *14* | *57* | *29* |
| — died (n) | 6 | 1 | 2 | 2 |
| — culled (n) | 1 | — | 2 | — |
| Died or culled at (days of trial) | 9<br>12<br>15<br>17<br>19<br>27<br>29 | 21 | 15<br>15<br>20<br>23 | 15<br>34 |

* day 15 - 19

noon. Also, in the trial house temperatures did not fall very often below 30°C, especially during tiamulin administration (outdoor temperatures of about 35°C), which apart from that depressed water intake.

### 4.3.2 Growth Performance

Mean weight of birds in each treatment at the beginning of the trial was 54 g. After 2 weeks of trial the birds achieved 547, 545, 542 and 541 g for groups 1, 2, 3, and 4, respectively (Table 30 and appendix 13). This situation drastically changed when tiamulin was administered for a period of 5 days to the birds during third week ($15^{th}$ to $19^{th}$ day), also shown in figure 13. Tiamulin co-administered with semduramicin (4) caused significantly ($p<0.05$) lower (937 g) weight than groups 1 (1003 g), 2 (996 g) and 3 (994 g) calculated after 3 weeks. The 5 days co-administration of tiamulin with semduramicin considerable depression of weight at the week when tiamulin was administered. This effect was still reflected after $4^{th}$ week of age by statistically lower weight (1458 g) gained in co-administered group 4, however similar to control (1497). The tiamulin treated group 2 (1520 g) and semduramicin group 3 (1527 g) revealed higher values than co-administered group 4. At the end of the trial 2062, 2067, 2084 and 2008 g body weight was measured in groups 1, 2, 3 and 4, respectively ($p>0.05$). Moreover when considered the ultimate achieved weight of all live birds per pen then the numerical value of all experimental groups (16,347, 15,828, and 15,669 g in groups 2, 3, and 4, respectively) were higher than in the control (15,083 g).

Values for weekly weight gain proved the existence of incompatibility only when tiamulin used in drinking water for a period of 5 days at third week (appendix 14). In that week incompatibility of tiamulin and semduramicin caused lower weight gain in group 4 showing

396 g in comparison to other groups with 456, 451 and 453 g in control, the tiamulin and the semduramicin group, respectively. In all other weeks birds gained similarly irrespective to all groups.

*Table 30.* ***Growth performance (LWG and FI) and FCE (gLWG $Kg^{-1}$FI) during co-administration of tiamulin and semduramicin in broiler chicks (54 g at start)***

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| **Semduramicin** (mg $kg^{-1}$ feed) | — | — | **25** | **25** |
| **Tiamulin** (mg $L^{-1}$ water) 5 days* | — | **250** | — | **250** |
| No. of replicates (cages) | 10 | 10 | 10 | 10 |
| Chicks per replicate | 8 | 8 | 8 | 8 |
| Final LW, (±sd) | 2062 (±89) | 2067 (±72) | 2084 (±58) | 2008 (±70) |
| *Relative to (1)* | 100 | 100 | 101 | 97 |
| Total LWG, (±sd) | 2008 (±89) | 2013 (±72) | 2030 (±58) | 1954 (±70) |
| *Relative to (1)* | 100 | 100 | 101 | 97 |
| Total FI, (±sd) | $3018^{a}$ (±69) | $3009^{a}$ (±115) | $2996^{a}$ (±117) | $2877^{b}$ (±93) |
| *Relative to (1)* | 100 | 100 | 99 | 95 |
| FCE, (±sd) | 665 (±20) | 669 (±22) | 678 (±19) | 679 (±16) |
| *Relative to (1)* | 100 | 101 | 102 | 102 |

* day 15 - 19

**$^{ab}$Different superscripts in the same row differ significantly (p<0.05)**

Figure 13 shows cumulative weight gains for the period 1st plus 2nd week, 3rd plus 4th week, 5th week as well as 1st to 5th week. As far as weight gain is concerned, the incompatibility of semduramicin and tiamulin reached significance only for the period 3rd plus 4th week. But considering only weekly intervals, the incompatibility was expressed only in week 3, during tiamulin administration.

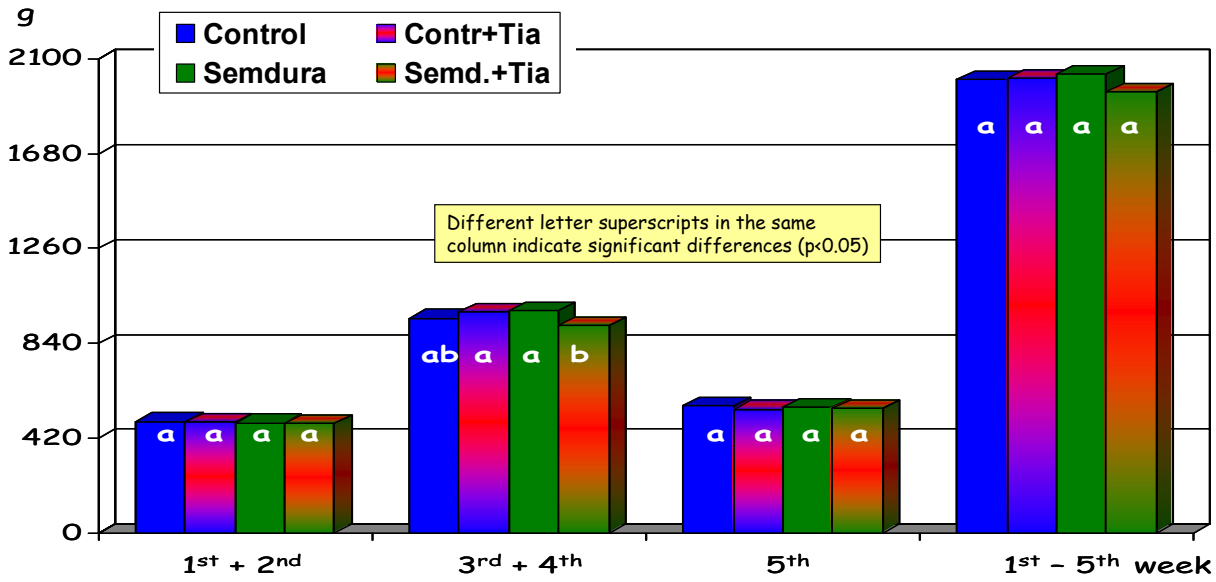


*Figure 13.* ***Live weight gain in different periods of the trial and cumulative live weight gain of female broilers, experiment 3***

### 4.3.3 Feed Intake

The feed consumption in different weeks showed that all groups consumed similar amounts of feed until 3rd week before administration of tiamulin in drinking water was started. But, tiamulin caused drastic depression in feed intake during co-administration with semduramicin at third week. In that week group (4) consumed 558 g, which was lower than control (617 g), tiamulin group (597 g) and semduramicin group (607 g) ($p<0.05$). This interaction also continued for the next week. But at the last week of trial statistically non different ($p>0.05$) feed intake data could be observed with 1023, 1013, 1006 and 981 g in groups 1, 2, 3, and 4, respectively (appendix 15).

The feed intake depression in the semudaramicin/tiamulin group during week 3 (5 days coadministration) and week 4 was as severe, that the cumulative feed intake of this group for the entire trial was significantly lower than for all other groups (Figure 14).

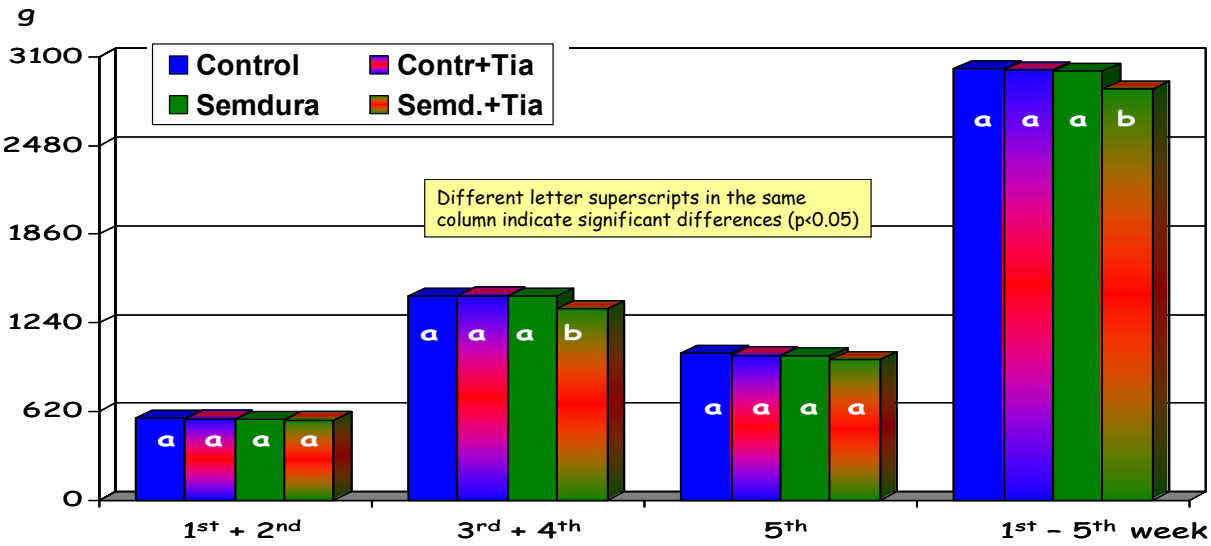


*Figure 14.* ***Feed intake in different periods of the trial and cumlative feed intake of female broilers, experiment 3***

### 4. 3. 4 Feed Conversion Efficiency

Feed conversion efficiency (g LWG $kg^{-1}$ FI) did not differ significantly before tiamulin administration until the age of 2 weeks. But during tiamulin administration feed efficiency decreased significantly (710 g LWG $kg^{-1}$ FI) compared to all other groups (control (740), tiamulin alone (756) and semduramicin alone (746 g LWG $kg^{-1}$ FI)). In week 4, reduced feed intake of the semduramicin/tiamulin group and a less reduced gain lead to better feed efficiency figure for this group (668 significantly better than the control value 613 g LWG $kg^{-1}$ FI). In the last week of trial the values for all groups did not indicate any differences (appendix 16).

Poor feed efficiency in week 3 and better feed efficiency in wee 4 resulted in the absence of significant differences for the period wee 3 plus week 4 for the smeduramicin/tiamulin group (Figure 15). At completion of the trial the semduramicin/itamulin group showed numerically the best feed efficiency, however not significant ($p>0.05$).

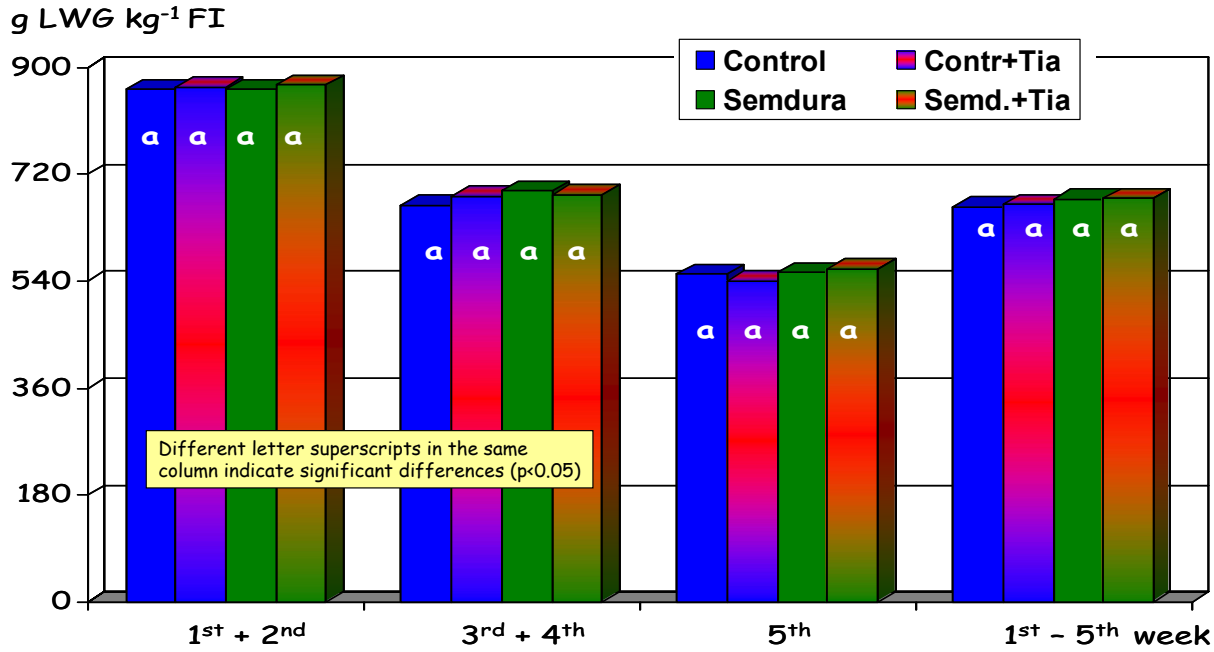


*Figure 15.* ***Feed efficiency in different periods of the trial and cumulative feed efficiency of female broilers, experiment 3***

### 4.3.5 Daily Water and Feed Intake During 15th to 21st Day

The effect of tiamulin on water consumption in was distinct from first day of administration (Figure 16 and Appendix 17). Tiamulin caused lower water consumption of 137 g and 112 g in groups (2) and coadministered group (4) already at first day (15th day) of co-administration ($p<0.05$). Water consumption was higher ($p<0.05$) in control (175 g) as well as semduramicin group (178 g). Two days later (day 17), the tiamulin group showed a tendency to recover, although water intake (202 g) was still significantly lower than in the control (237 g) and in the semduramicin group 3 (243 g), but it was significantly

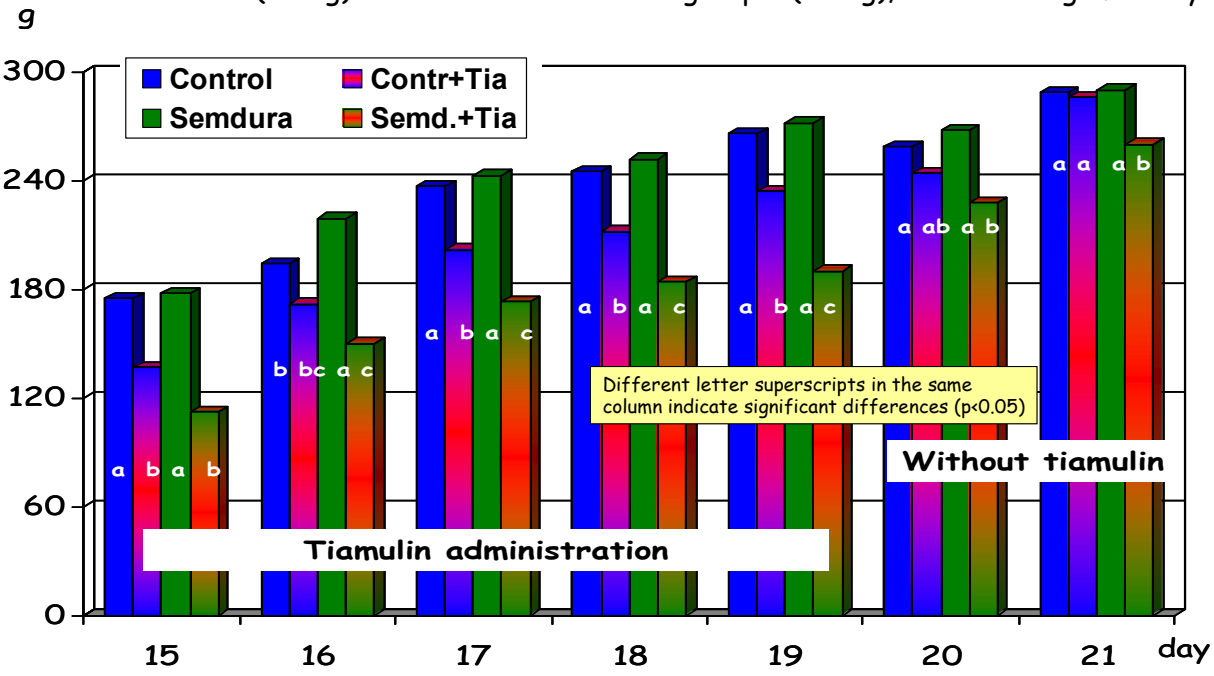


*Figure 16.* ***Daily water intake (g/bird) of female broilers during 5 days of tiamulin administration (day 15-19) and in the subsequent 2 days***

higher than in the semduramcin/tiamlunin group 4 (173 g). These differences remained more or less stable including day 19, the last day of tiamulin administration (Figure 16). For the entire period of taimulin administration (days 15-19), the control and the semduramicin group showed comparable cumulative water intake quantities (1117 and 1164 g, respectively), whereas the tiamulin consumed significantly less water (957 g), the semduramicin/tiamulin group showed the significantly lowest intake (809 g).

In the remaining 2 days of the 3rd week, no significant difference could be observed anymore between the control, the tiamulin and the semduramicin groups (cumulative water intake 548, 530, and 558 g, respectively), only the semduramicin/tiamulin group consumed also in this (tiamulin free) period significantly ($p<0.05$) less water (488 g).

Feed consumption was comparably affected by simultaneous tiamulin as water intake. Already on the first day, feed intake of the semduramicin/tiamulin group was significantly lower (73 g) than the other groups (control 84 g), tiamulin (80 g), semduramicin (82 g). This situation continued until 5th day of that week (Appendix 18 and Figure 17). Despite the significantly reduced water intake of the tiamulin group during this period, feed consumption was only marginally affected ($p>0.05$). Cumulative data for that period (424, 415, 418, and 376 g for the groups 1, 2,3, and 4, respectively) indicate smaller differences in feed intake than in water consumption. Whereas water intake in the semduramicin/tiamulin group was reduced by 28 %, feed intake decreased only by 11 %, compared to the control. On subsequent 6th day the semduramicin/taimulin group (4) tried to compensate for the previous lower feed consumption showing similar values as control ($p>0.05$). But residual effects existed at 7th day of tiamulin treatment week showing again a significantly lower daily feed intake in the group 4. Cumulative results (tiamulin free days 20 and 21) indicate a more or less equal, at least insignificant difference in feed intake of all groups (194, 182, 189, and 181 g for groups 1,2,3, and 4. respectively).

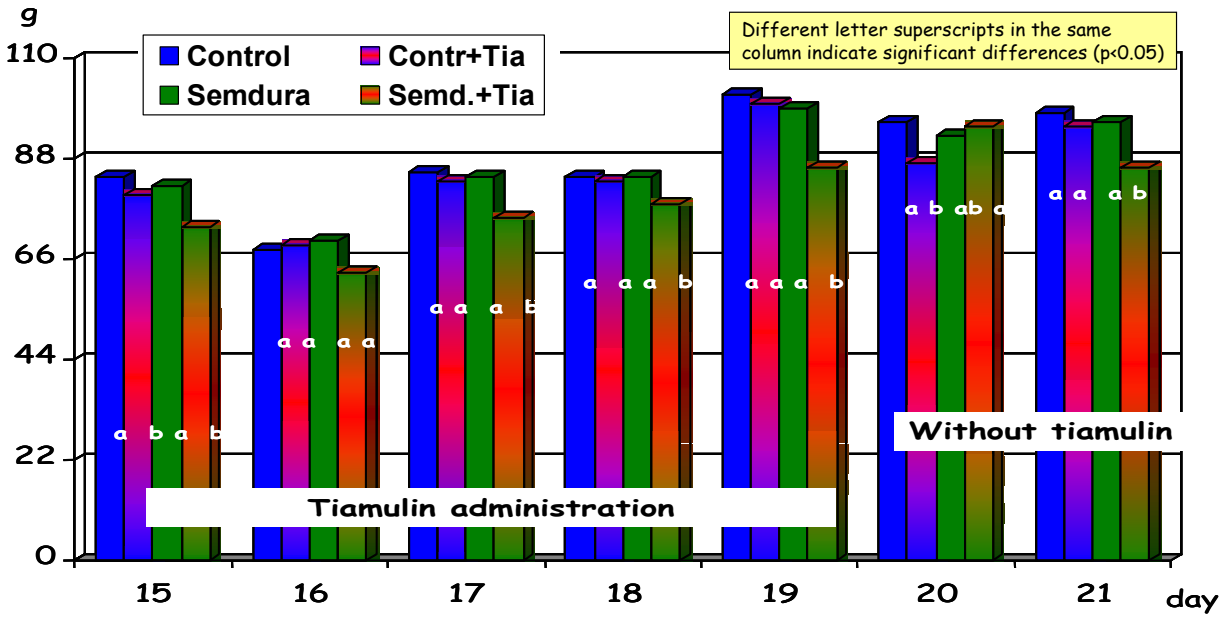


*Figure 17.* ***Daily feed intake (g/bird) of female broilers during 5 days of tiamulin administration (day 15-19) and in the subsequent 2 days***

Adverse effect on water consumption as well as feed intake due to coadministration of tiamulin and semduramicin also caused lower weight gain in the same week reflected in table 31. Chicks of the groups 2 and 4 showed in week 4 a considerably but insignificant higher weight gain than birds of the control group (see Table 31). Relating the weight gain to body weight at beginning of the respective week, the compensatory growth becomes clear. In the third week, also the relative weight gain of the semudramicin/taimulin group was significantly lower than that of the control group (73 versus 84 %, $p<0.05$), whereas in the fourth week (56 versus 49 %, $p<0.05$) and the period fourth to fifth wee inverse relations were observed (115 versus 106 %, $p<0.05$, Table 31).

Table 31. ***Body weight gain of broiler chicks as influenced by co-administration of tiamulin and semduramicin***

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** | **SEM** |
|---|---|---|---|---|---|
| **Semduramicin** (mg $kg^{-1}$ feed) | — | — | **25** | **25** | |
| **Tiamulin** (mg $L^{-1}$ water) 5 days* | — | **250** | — | **250** | |
| No. of replicates (cages) | 10 | 10 | 10 | 10 | |
| Chicks per replicate | 8 | 8 | 8 | 8 | |
| Day 15-21 (g) | $456^{a}$ | $451^{a}$ | $453^{a}$ | $396^{b}$ | 5.1 |
| — % gain of bw 14 | $83.5^{a}$ | $82.9^{a}$ | $83.7^{a}$ | $73.3^{b}$ | 0.09 |
| Day 22-28 (g) | $494^{a}$ | $524^{a}$ | $533^{a}$ | $522^{a}$ | 6.1 |
| — % gain of bw 21 | $49.3^{b}$ | $52.7^{ab}$ | $53.6^{a}$ | $55.7^{a}$ | 0.66 |
| Day 29-35 (g) | $564^{a}$ | $547^{a}$ | $557^{a}$ | $550^{a}$ | 5.0 |
| — % gain of bw 28 | $37.7^{a}$ | $36.0^{a}$ | $36.5^{a}$ | $37.8^{a}$ | 0.32 |
| Day 22-35 (g) | $1059^{a}$ | $1071^{a}$ | $1090^{a}$ | $1072^{a}$ | 8.9 |
| — % gain of bw 21 | $105.5^{b}$ | $107.6^{b}$ | $109.6^{ab}$ | $114.5^{a}$ | 0.99 |

* day 15 - 19

**$^{ab}$Different superscripts in the same row differ significantly ($p<0.05$)**

Nevertheless, the depressive effect of the preceding administration of tiamulin and semduramicin on feed intake of group 4 was still present (Figure 16). In summary, the performance data of the third (and fourth) week suggest an interaction between tiamulin and semduramicin resulting in a temporary depression of feed intake and growth, which could not fully be compensated during the five weeks trial.

### 4.3.6 Blood Chemistry and Enzyme activities, Experiment 3

Dietary treatments evidently did not have any effect ($P>0.05$) on the concentration of protein, albumin and glucose in the plasma (Table 32). As expected, similar observations could be made for sodium and chloride, which are mainly located extra-cellular, whereas a slight tendency to increased plasma potassium concentrations ($P>0.05$) could be found in the groups 3 and 4 compared to the control (5.4 and 5.1 *versus* 4.9 mmol $L^{-1}$). Also for the creatine kinase and aspartate aminotransferase a trend to higher activities is evident in birds of group 3; on the other hand, activity of alkaline phosphatase seems to be reduced in this group. In contrast, chicks treated with tiamulin in groups 2 and 4 showed markedly reduced levels of a-glutamyl transferase (-8 and -9 %, $P>0.05$) and marginally elevated lactate dehydrogenase activities in comparison to the control birds

Table 32. ***Concentration of different and substrates and enzymes in plasma of broiler chicks, experiment 3***

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| **Semduramicin** (mg $kg^{-1}$ feed) | — | — | **25** | **25** |
| **Tiamulin** (mg $L^{-1}$ water) 5 days* | — | **250** | — | **250** |
| No. of chicks | 10 | 10 | 10 | 10 |
| Protein (g $L^{-1}$) | 36.0 | 34.6 | 34.4 | 33.6 |
| (±sd) | (4.4) | (3.2) | (3.1) | (3,6) |
| *Relative to (1)* | *100* | *96* | *95* | *93* |
| Albumine (g $L^{-1}$) | 13.7 | 13.8 | 13.7 | 13.6 |
| (±sd) | (1.0) | (1.4) | (1.1) | (1.9) |
| *Relative to (1)* | *100* | *100* | *98* | *98* |
| Glucose ( mmol $L^{-1}$), | 13.64 | 13.65 | 13.38 | 13.41 |
| (±sd) | (0.66) | (1.37) | (0.97) | (0.78) |
| *Relative to (1)* | *100* | *100* | *98* | *98* |
| Uric acid (µmol $L^{-1}$) | 370 | 351 | 399 | 378 |
| (±sd) | (103) | (110) | (139) | (171) |
| *Relative to (1)* | *100* | *95* | *108* | *102* |
| Sodium (mmol $L^{-1}$) | 157 | 156 | 157 | 157 |
| (±sd) | (3) | (3) | (2) | (2) |
| *Relative to (1)* | *100* | *99* | *100* | *100* |
| Chloride (mmol $L^{-1}$) | 116.0 | 116.0 | 116.0 | 116.0 |
| (±sd) | (2.6) | (2.6) | (2.3) | (3.1) |
| *Relative to (1)* | *100* | *100* | *100* | *100* |
| Potassium (mmol $L^{-1}$) | 4.9 | 4.7 | 5.4 | 5.1 |
| (±sd) | (0.5) | (1.4) | (1.0) | (0.8) |
| *Relative to (1)* | *100* | *97* | *110* | *104* |
| Creatine kinase (U $L^{-1}$) | 12,091 | 11,879 | 14,088 | 12,355 |
| (±sd) | (6,808) | (3,824) | (7,445) | (7,722) |
| *Relative to (1)* | *100* | *98* | *117* | *102* |
| Alkaline phosphatase (U $L^{-1}$) | 2,935 | 2,946 | 2,396 | 2,613 |
| (±sd) | 797 | 695 | 470 | 562 |
| *Relative to (1)* | *100* | *100* | *82* | *89* |
| Lactate dehydrogenase (U $L^{-1}$) | 1,116 | 1,160 | 1,097 | 1,177 |
| (±sd) | 358 | 268 | 275 | 628 |
| *Relative to (1)* | *100* | *104* | *98* | *105* |
| Aspartate aminotransferase (U $L^{-1}$) | 264.9 | 262.0 | 291.7 | 265.8 |
| (±sd) | 56.9 | 46.0 | 72.4 | 65.3 |
| *Relative to (1)* | *100* | *99* | *110* | *100* |
| γ-Glutamyl transferase (U $L^{-1}$) | 21.0 | 19.4 | 20.8 | 19.2 |
| (±sd) | 4.6 | 4.3 | 4.2 | 3.7 |
| *Relative to (1)* | *100* | *92* | *99* | *91* |
| Alanine aminotransferase (U $L^{-1}$) | 4.8 | 4.5 | 5.0 | 4.9 |
| (±sd) | (1.8) | (0.7) | (1.6) | (1.5) |
| *Relative to (1)* | *100* | *91* | *104* | *103* |

* day 15 – 19

All parameters did not show any significant differences ($p>0.05$), ANOVA, Tukey-B-Test

*Table 33.* ***Blood count in broiler chicks at day 36***

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| **Semduramicin** (mg $kg^{-1}$ feed) | — | — | **25** | **25** |
| **Tiamulin** (mg $L^{-1}$ water) 5 days* | — | **250** | — | **250** |
| No. of chicks | 10 | 10 | 10 | 10 |
| Haematocrit (%) | 29.3 | 30.4 | 30.2 | 29.9 |
| (±sd) | (3.1) | (1.9) | (3.5) | (4.1) |
| *Relative to (1)* | *100* | *104* | *103* | *102* |
| Erythrocytes (n$x10^6$g $\mu L^{-1}$) | 2.48 | 2.26 | 2.38 | 2.30 |
| (±sd) | (0.45) | (0.25) | (0.34) | (0.17) |
| *Relative to (1)* | *100* | *91* | *96* | *93* |
| Leucocytes (n $\mu L^{-1}$), | 12312 | 12888 | 14120 | 15494 |
| (±sd) | (1505) | (4386) | (3122) | (3309) |
| *Relative to (1)* | *100* | *105* | *115* | *126* |
| Heterophils (%) | 36 | 29 | 29 | 25 |
| (±sd) | (12) | (11) | (7) | (13) |
| *Relative to (1)* | *100* | *79* | *79* | *70* |
| Lymphocytes (%) | 61 | 70 | 70 | 73 |
| (±sd) | (12) | (11) | (7) | (13) |
| *Relative to (1)* | *100* | *114* | *114* | *118* |
| Heterophils/Lymphozytes | 0.67 | 0.44 | 0.42 | 0.39 |
| (±sd) | (0.46) | (0.22) | (0.14) | (0.27) |
| *Relative to (1)* | *100* | *66* | *63* | *58* |
| Basophiles (%) | 0.2 | 0.2 | 0.4 | 0.9 |
| (±sd) | (0.6) | (0.4) | (1.0) | (1.1) |
| *Relative to (1)* | *100* | *100* | *200* | *450* |
| Eosinophiles (%) | 1.0 | 0.5 | 0.7 | 0.5 |
| (±sd) | (2.8) | (1.3) | (1.1) | (0.7) |
| *Relative to (1)* | *100* | *50* | *70* | *50* |
| Monocytes (%) | 1.3 | 0.9 | 0.6 | 0.7 |
| (±sd) | (0.9) | (0.7) | (0.7) | (1.1) |
| *Relative to (1)* | *100* | *69* | *46* | *54* |

* day 15 - 19

[ab]Values with different superscript in the same row are different ($P<0.05$)

(+4 and +5 %). Results for enzyme activities seem to be somewhat inconsistent concerning trend and extent of the observed alterations. Moreover, mean values of the enzyme activities were accompanied by exceptionally high standard deviations or coefficients of variations, particularly for the CK with values between 0.32 and 0.63. In summary, the differences found however not significant can obviously not be attributed to the treatment.

Haematocrit and number of erythrocytes did not differ between treatment groups. Although, from group (1) to (4) a markedly increasing number of leukocytes could be found (12,312, 12,888, 14,120 and 15,494 $\mu L^{-1}$), differences between the treatment groups were not significant, probably also because of the relatively high standard

deviations (Table 33). However, the analysis for main effects and interactions (semduramicin x tiamulin) by 2-way-ANOVA provides a significant effect of semduramicin on leukocytes, but no interaction. In correspondence with the leukocytes proportions of lymphocytes showed a similar trend, whereas for heterophils a reverse behaviour could be observed. The H-/L-ratio, the quotient between heterophils and lymphocytes, which is an indication for stress response – as lower the values as milder the reaction to the environmental stress - was clearly reduced in groups (2) to (4) compared to control (0.44, 4.42 and 0.39 *vs* 0.67; P > 0.05). It could be therefore supposed that the control group was more stressed, probably by infections, than the groups receiving semduramicin and tiamulin.

### 4.3.7 Pathology of Selected Birds After Feeding Trial

The *pathological-anatomical (macroscopical)* examination of 40 birds at day 36 revealed for almost all animals no specific findings (Table 34). In some chicks acute haemorrhages of obviously traumatic origin were found, which however might be attributed to the handling of the birds or to manipulations during blood sampling. Other observations had to be assessed as coincidental findings, which did not show any treatment related frequency distribution.

***Table 34.*** *Pathological –anatomical diagnosis (macroscopic examination) of 40(10 per group) at 36 days of old broiler chicks*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| **Semduramicin** (mg $kg^{-1}$ feed) | — | — | **25** | **25** |
| **Tiamulin** (mg $L^{-1}$ water) 5 days* | — | **250** | — | **250** |
| No. of chicks | 10 | 10 | 10 | 10 |
| Negative findings | 7 | 6 | 6 | 6 |
| Breast blister | — | 1 | 1 | — |
| Mild muscle haemorrhages | 2 | — | 1 | 2 |
| Atrophy of stomach wall | 1 | — | — | — |
| Mild splenomegalia | — | — | — | 1 |
| Moderate splenomegalia | — | 1 | 1 | — |
| Moderate subepicardial haemorrhages | — | 1 | — | — |
| Mild hydropericardium | — | 1 | 1 | — |
| Severe hydropericardium | — | — | 1 | — |
| Moderate haemopericardium | — | — | — | 1 |
| Dsychondropathy of tibia | — | 1 | — | — |
| Fracture of left tibia | — | — | — | 1 |

* day 15 – 19

NE- not examined; Birds of group (2) and (3) should only be examined in case of strong indications for treatment effects after examination of the chicks of group (1) and (2)

The pathological-histological (light-microscopical) examinations were performed on 10 birds each of group 1 and 4) shown in Tables 34 and 35. With the procedures applied no

***Table 35.*** *Pathological-histological diagnoses (light-microscopic examination) of 40 (10 per group) broiler chicks*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| **Semduramicin** (mg $kg^{-1}$ feed) | — | — | **25** | **25** |
| **Tiamulin** (mg $L^{-1}$ water) 5 days* | — | **250** | — | **250** |
| Multifocal, acute muscle degeneration, mild symptoms | 5 | NE | NE | 5 |
| Isolated, acute muscle degeneration, mild symptoms | 3 | NE | NE | 4 |
| Acute muscle degeneration without inflammation | 2 | NE | NE | 1 |
| Mild fatty infiltration into hepatocytes | 2 | NE | NE | 6 |
| Mild cardiac haemorrhages | 1 | NE | NE | 1 |
| Mild non-purulent inflammations of liver, kidney and/or heart | 4 | NE | NE | 3 |
| Muscle degeneration – mild symptoms, inflammatory reactions | — | NE | NE | 3 |
| Myocarditis | — | NE | NE | 1 |
| No glycogen in hepatoctes | 2 | NE | NE | 2 |
| Low glycogen in hepatozytes | 2 | NE | NE | 1 |
| Medium glycogen in hepatozeytes | 2 | NE | NE | 3 |
| High glycogen in hepatocytes | 4 | NE | NE | 3 |

* day 15 - 19

NE- not examined; Birds of group (2) and (3) should only be examined in case of strong indications for treatment effects after examination of the chicks of group (1) and (2)

evidence for specific group differences was found which could be traced back to the treatment during growth trial. However, in birds of group 4 fatty infiltrations of hepatocytes occurred slightly more frequent than in the control. The relevance of these findings is not clear, particularly because glycogen content in the hepatocytes did not differ between the treatment groups. There are also no indications for an increased susceptibility of group 4 to myopathy.

In summary, clear indications for treatment effects could not be found, neither with the pathological-anatomical examination (Table 34), nor with the conventional histo-pathological light-microscopy (Table 35); particularly, most of the findings could not be attributed to the substances (semduramicin, tiamulin) administered and coadministered during the growth trial.

## 4.4 Growth and Carcass Characteristics of broilers after 5-8 days Withdrawal of Semduramicin

### 4.4.1 Carcass Traits

At the end of extended fattening period broiler of the former semduramycin group showed a slightly higher final body weight than the birds of the control group (2569 vs 2477g, P>0.05) as shown in Table 36 and Appendix 19. Accordingly, daily weight gain per pen was about 16% higher in the semduramicin group (3); also the higher hot carcass weight of the semduramicin birds (+4%; P>0.05) reflects completely the higher final weight (Table 36). Nevertheless, a significant effect (P>0.05) of the former dietary treatments on the main slaughter characteristics determined immediately after slaughtering (without cooling) could not be observed, neither for the absolute nor for

the percentage figures; only gizzard related to LW (%) was significantly (P<0.05) lower in the semduramicin group, which should not be a relevant finding because of the low absolute weight of the gizzard. However, in the semduramicin group also a tendency to lower liver weight related to LW is evident.

*Table 36.* ***Performance data of the control and the semduramicin group during 5-8 days withdrawal***

| **Code** | | **(1)** | **(3)** |
|---|---|---|---|
| Semduramycin (mg $kg^{-1}$ feed), | Day 1-35 | — | 25 |
| | Day 36-43 | — | — |
| LW, selected birds (g) at day 35, (±sd) | | 2085 (±112) | 2114 (±62) |
| *Relative to (1)* | | *100* | *101* |
| LW at slaughter (g), (±sd) | | 2477 (±178) | 2569 (±168) |
| *Relative to (1)* | | *100* | *104* |
| LWG $day^{-1}$ (day 35 to slaughter), (±sd) | | 60 (±9) | 70 (±10) |
| *Relative to (1)* | | *100* | *116* |

[a]All the parameters showed insignificant differences at 5 % level, ANOVA, Tukey B Test

*Table 37.* ***Weight of slaughtered birds and their different parts (in gram and per cent of live bird) immediately after slaughtering after 5-8 days withdrawal of semduramicin***

| **Code** | **(1)** | **(3)** | **(1)** | **(3)** |
|---|---|---|---|---|
| Semduramycin, Day 1-35 | — | 25mg $kg^{-1}$ | — | 25mg $kg^{-1}$ |
| Day 36-43 | — | — | — | — |
| | **Weight (in gram)** | | ***Weight (% of LW)*** | |
| Hot carcass[#], (±sd) | 1868 (164) | 1951 (154) | 75.4 (2.4) | 75.9 (2.0) |
| *Relative to (1)* | *100* | *104* | *100* | *101* |
| Shanks, (±sd) | 82.4 (8.6) | 84.6 (4.5) | 3.33 (0.28) | 3.30 (0.22) |
| *Relative to (1)* | *100* | *103* | *100* | *99* |
| Head and neck, (±sd) | 118 (22) | 122 (21) | 4.79 (0.93) | 4.78 (0.84) |
| *Relative to (1)* | *100* | *104* | *100* | *100* |
| Viscera[§], (±sd) | 277 (29) | 277 (30) | 11.21 (1.18) | 10.79 (0.91) |
| *Relative to (1)* | *100* | *100* | *100* | *96* |
| Liver*, (±sd) | 55.9 (10.1) | 54.8 (8.8) | 2.26 (0.37) | 2.13 (0.28) |
| *Relative to (1)* | *100* | *98* | *100* | *94* |
| Heart, (±sd) | 10.4 (1.4) | 10.9 (1.0) | 0.42 (0.05) | 0.42 (0.05) |
| *Relative to (1)* | *100* | *105* | *100* | *101* |
| Gizzard, (±sd) | 21.0 (3.2) | 19.7 (2.7) | 0.85[a] (0.14) | 0.77[b] (0.10) |
| *Relative to (1)* | *100* | *94* | *100* | *90* |

[#]Eviscerated [§] Viscera: Stomach, intestine, liver, spleen, lung and heart *Without gallbladder

[ab] Values with different superscript in the same parameter in same row are different (P<0.05)

After an overnight cooling almost similar results were obtained as for the fresh material (Appendix 21). However, as expected in both treatment groups carcass weight, dressing percentage and weight of liver, heart and gizzard was somewhat lower after the overnight storage, because of dripping off losses.

In the Appendix 22 the carcass parts given as absolute and relative figures (% of eviscerated carcass with giblets) for breasts, legs and wings with the corresponding portions of skin, muscle and fat are presented. According to LW at slaughter weight of breast, legs and wings was higher in the withdrawal birds, nevertheless, significant differences ($P<0.05$) between control and withdrawal group could only be observed for total breast muscle (460.9 vs 495.1g; + 7%) and total muscles of breast, legs and thighs (817.8vs 870.1g;+ 6%). However, differences between treatment groups considerably decrease when carcass traits were related to eviscerated carcass (2-3%; $P>0.05$).

### 4.4.2 Chemical composition of edible parts of carcass

Although the intra-muscular fat content in the meat of the semduramicin fed broilers was significantly higher ($P<0.05$) than in control chicks, absolute group differences were small. Moreover the low absolute values were accompanied by high standard deviations (Table 38). From this point of view the results should not be overestimated. The crude protein content did not differ between treatment groups.

*Table 38.* ***Chemical composition (g/100 g fresh matter) of breast muscle meat from the carcass after overnight chilling***

| **Code** | **(1)** | **(3)** |
|---|---|---|
| Semduramycin (mg $kg^{-1}$ feed), Day 1-35 | — | 25 |
| Day 36-43 | — | — |
| Carcass weight* (g), (±sd) | $1934^{a}$ (±166) | $2015^{a}$ (±157) |
| *Relative to (1)* | *100* | *104* |
| Crude protein, (±sd) | $23.1^{a}$ (±0.8) | $23.4^{a}$ (±0.4) |
| *Relative to (1)* | *100* | *102* |
| Crude fat, (±sd) | $1.57^{a}$ (±0.61) | $2.01^{b}$ (±0.60) |
| *Relative to (1)* | *100* | *135* |

*Eviscerated with giblet; bw- body weight;
$^{ab}$Values with different superscript in the same row are different ($P<0.05$)

### 4.4.3 Sensory Characteristics

In general, with average values for juiciness, tenderness, aroma/flavour and general impression of 5.1, 5.1, 4.2 and 4.6 (Appendix 23 and 24), respectively, and the absence of unpleasant, pungent flavour, the sensory characteristics of the slaughtered birds could be assessed as very good, independent of the treatment group (Appendix 23 and 24). The aroma and flavour of the semduramicin broilers was evidently more favourable ($P<0.05$) in comparison to the control birds (4.5 versus 4.0); accordingly, there was also a tendency to an improvement ($P<0.10$) of the general impression (4.7 versus 4.5). Both findings may be a result of the slightly higher intramuscularly fat content in the breast muscle of the semduramicin birds.

Juiciness and tenderness were not influenced by the former dietary treatment. Although an unpleasant pungent flavour could not be observed in any case, in 7 broilers of the control group and 6 broilers of the semduramicin group a slightly sour aroma was identified by some panel members.

Also, in 3 birds of control and 5 chicks of semduramicin group a metallic or tasteless (1 bird of semduramicin group) aroma was detected (appendix 25). It seems that the unpleasant taste affected only in case of the semduramicin birds the scores for aroma and flavour, because the affected broilers (n=9) of the control and semduramicin group (n=8) had an average aroma/flavour score of 4.0±0.4 and 4.0±0.9 respectively.

# 5. Discussion

The three studies performed on different additives will be discussed separately.

## 5.1 Fumaric Acid

Fumaric acid is approved at EU statutory level as a preservative, to protect cereals and other feedstuffs from spoilage by bacteria and fungi. In the study presented FA has been tested under conditions usually applied for a growth promoting feed additive. This corresponds to the feeding practice in field, where FA and other organic acids are added to diets (mainly for pigs) more or less as a substitute for growth promoting antimicrobial substances.

### 5.1.1 Growth, Feed Consumption and Feed Efficiency

Addition of 1.25 % FA in feed caused higher ($p<0.05$) weight gain but 2.5, and 3.75 % FA reflected growth only similar to control value ($p>0.05$). It could be concluded that there might be only a small optimum dose range for FA in broiler diets and that lower dosage than the lowest investigated may also be effective as 2.5 % was not. But the diets used in the experiment contained cereals of good quality a dose response to FA may appear different from that shown, if feedstuffs of minor quality had been used.

Above positive effects were proved by many scientists but the levels of inclusion are important to discuss. Eidelsburger and Kirchgessner, 1994, demonstrated that up to 1.0 % FA has a positive effect in broilers, which is in agreement with our results. Patten and Waldroup, 1988, found that 1.0 % FA in broiler resulted in higher body weight gain but did not influence feed utilization. Our studies showed also this lack of an significant effect on feed efficiency. Grassmann and Klasna, 1986, also confirmed this result for the rat. Tschierschwitz *et al.,* 1982, demonstrated that the addition of FA at 2 % levels improved the utilization of the nutrient as a result of elevated enzyme activities in rat. Vogt *et al.,* 1981, reported that FA significantly improved feed utilization curvilinearly, with the peak response occurring at an inclusion rate between 1.0 and 1.5%. This result is regarded as a confirmation of our above conclusion that in broiler nutrition, also lower levels of FA than tested (1.25 %) should be taken into consideration.

Another fact should not be neglected. Poultry at birth has a digestive system capable of digesting solid feeds. So, HCl and specific enzyme secretion are functional from hatching. Hydrocholoric acid and pepsinogen are secreted in the proventriculus, in quantities much larger than in mammals (Moran, 1982) and on a continuous basis. The high HCl production capacity of poultry makes the classical acidification strategy used in piglets questionable. Lower amounts of an organic acid may suffice to create the same effects (same magnitude of improvements in growth) in poultry as needed for piglets. As a matter of fact, the optimal dosage for piglets is about 2 % and higher (Kirchgessner and Roth, 1976).

Kirchgessner and Roth, 1976, found that an addition of 2.0 % FA increased weight gains of piglets by 11.6 %. Adding 4.0 % FA lead to an increase of only 3.4. A comparable narrow range for the optimum dose is also assumed for broilers base

on our findings. On the other hand, 2.5 and 3.75 % FA may cause a lowering of gastric pH below optimum conditions for pepsin functioning. The FA might cause increased permeability of the epithelial cells permitting higher amount of FA entering the body. Studying membrane vesicles, Voinova *et al.,* 1987, found FA significantly increasing pH activity with all substrates investigated. Higher than optimum FA might interfere with the intermediary metabolism deteriorating utilization of nutrients already absorbed. These types of negative effects might hinder the general positive effect to appear which are obvious at lower FA inclusion amounts (e.g. 1.25 %).

The reason for the beneficial FA effect in poultry can be seen in a multitude of described influences on digestion, absorption and utilization of the nutrients resulting also in health protecting effect. But own studies on the mode of action of FA were not conducted.

Dietary acidification caused lowering the pH (Blank *et al,* 1999) created better environment for pepsin to work upon protein by increasing gastric proteolysis and amino acid digestibility (Vogt *et al.,* 1981; Blank *et al.,* 1999; Taylor, 1959; Taylor, 1962). The acid anion has been shown to complex with Ca, P, Mg, and Zn, which results in an improved digestibility of these minerals. Furthermore, organic acid serves as substrates in the intermediary metabolism (Kirchgessner and Roth, 1988; Giesting *et al.,* 1991), resulting in subsequent better utilization to stimulate body syntheses as body mass and egg production (Okolelova and Krivoruchko, 1991). Other scientist focus on the antimicrobial property of FA reducing the microbial count in the GIT and causing therefore beneficial effects like an antibiotic growth promoter (Vogt *et al.*, 1981). Moreover, Blank *et al.,* 1999, could demonstrate that FA, as a readily available energy source, has a local tropic affect on the mucosa of the small intestine in weanling pigs. This mode of action should also be considered for poultry.

The concentrations of VFA in gastrointestinal tract of avian species and very high in the caeca are mainly acetate, but some propionate and butyrate. Also VFAs are one of the by-products of microbial decomposition of uric acid (Braun and Campbell, 1989). Levels of VFAs are higher in the portal blood of conventionally raised compared to germ-free birds, suggesting that VFAs formed by bacteria. They are absorbed from both the small intestine and the caeca by passive transport (Sudo and Duke, 1980). Since the concentration of VFAs is higher inside the lumen, absorption occurs by the passive movement of these compounds down their concentration gradient. While the rate of absorption of propionate and butyrate is equal in the small intestine and the caeca, acetate is absorbed faster in the caeca. So, in our experiment higher level (5.0 and 7.5%) of FA might have caused lowering of the pH (cited earlier in chapter 2), which in turn reduces the multiplication of micro-organisms in GIT and ultimately depresses VFA production. Inefficient energy utilization and imbalance of the energy protein ratio may be the result. Microbial fermentation in the caeca of the ptarmigan can provide VFAs to meet 11-18% of the energy requirement for basal metabolic rate (Gasaway, 1976).

Feeds low in digestibility are thought to place constraints on DMI because of their slow clearance from the rumen and passage trough the digestive tract (Allen, 1996). DMI in non-ruminant animal is also limited by digestibility. There could be a connection between the low feed intake observed at 5.0 and 7.5 % FA in broiler diets and a more "bulky" character of that feed due to insufficient bacterial digestibility of nutrients (non starch polysaccharides). According to the conceptual framework for the metabolic-feedback theory (Illius and Jessop, 1996) an animal has a maximal productive capacity and a maximal rate at which nutrients can be used to meet productive requirements. When absorption of nutrients, principally protein and energy, exceeds requirements or when the ratio of nutrients absorbed is imbalanced, negative metabolic-feedback would impact DMI. By this theory a negative feedback between high amounts of FA and DMI has to be expected too. Kirchgessner *et al.,* 1991, indicated that up to 2.0% FA did not increase feed consumption in broiler as our experiment 2.5 and 3.75 % reflected.

On contrary, Bolduan, 1987, reported that at a low level of 1.2 % FA causes rapid emptying of stomach resulting in higher DM intake. So the higher feed intake could be expected on optimal (or low) dosages of FA, as observed in the experiment. Also unpublished studies (Gropp, 1974) suggest that also in piglets the growth promotion efficacy of FA is strongly correlated with an increased feed intake. Consequently, feed efficiency is only slightly improved by FA, if all.

Skinner *et al.,* 1991, demonstrated that the addition of FA (up to 0.25 %) significantly improved body weight of females and average weight gain of both sexes but found no effect on feed utilization of broilers. Feed consumption was significantly increased when diets contained 0.125 or 0.50 % FA. In another trial with male broilers the body weight was significantly ($P<0.05$) improved by the addition of 0.125 and 0.25 % FA. There were no significant differences in feed consumption; feed utilization was improved by the addition of all levels of FA.

In our experiment an inclusion up to 3.75%FA did not cause any reduction of FCE but only numerical value was higher in the 1.25 % FA group. This situation is in agreement with the results of Vogt *et al.,* 1981, indicating the FA curvelinerly improving feed efficiency (by 3.5 to 4.00 per cent) at an addition of 1 to 1.5 per cent. In other report by the same researchers (Vogt *et al.*, 1979) the inclusion of FA up to 8% caused significant improvement of feed efficiency by 1.3 to 7.3% in broiler, which is not in agreement with our result. Patten and Waldroup, 1988, reviewed that the inclusion of organic acids (including FA) up to the level of 2.0 % improves growth rate or feed utilization.

High FA (5.0 and 7.5 % in the diet) resulted in a severe significant depression of live weight gain, feed intake and feed efficiency (FCR only numerically depressed for 5.0 % FA). The highest level tolerated is 3.75 %. By this the level of margin for FA in broiler diets is 3 (3.75/1.25), considering 1.25 % FA as highest optimal dose. But if the presence of a growth promoting effect is considered as the criterion for exceeding the margin of safety, than this figure is considerably lower. The dosages applied in our studies as well as the (high) quality of feedstuffs not allow determining this value exactly.

### 5.1.2 Mortality, Health Status and Organ Weight

The mortality of birds was within the range of 0 to 4 % in all treatment groups, which is in the accepted limit of mortality in modern broiler production. Mortality figures seem rather accidentally distributed among the treatment groups so that mortality could be attributed to FA given in increasing amounts. Pathological report demonstrated no observation behind mortality but heart failure. Heart failure in rapidly growing broiler is common due to the unfavourable relation of the size of the heart and body mass. In faster growing broiler the heart does not grow according to other organs so that finally the relative size of the heart is lower (Olkowski, 2004). According to Skinner *et al.,* 1991, dietary FA had no adverse effects on mortality rate.

In our experiment inclusion up to the 5% level of FA did not cause any health problem apparently and after necropsy the observation was in agreement with Patten and Waldroup, 1988. They demonstrated that the inclusion of organic acids (including FA) up to the level of 2.0 % resulted in no health problems.

No kidney alterations have been observed in the control and in the 1.25 % FA group. The groups with 2.5 and 5.0 % showed one kidney (of 10) with capsulated blood, no discoloration in the 2.5 and 3.75 % FA group, but one in the 5 % group and 3 in the 7.5 % FA group. A very conservative assessment would at least not exclude a potential influence of FA on kidney function for these high dosages as known for humans (Kolbach and Nieboer, 1992).

## 5.2 Humic Acid

From the beginning of trial adverse effect on weight gain due to inclusion of humic acid in broiler ration especially at higher amounts became apparent. Subsequently a mild compensation could be observed. The results of Bailey *et al.*, 1996, are in agreement with our results. The authors fed Menefee® humate (MH) to male broilers, which did not affect body weight after 35 d. This may be due to much more sensitive epithelial layer of GIT of the day old chicken compared to older ones at least requiring an adjustment phase for high HA containing feed. It could also be identified from findings of Kocabağli *et al.*, 2002, which found highest adverse effects disappear promptly after two weeks. The study indicated that feeding 2.5 g HA (Farmagülatör DRY$^{TM}$Humate) kg$^{-1}$ complete feed from 22 to 42 days significantly improved body weight, which was 4.3 % greater than those of the control birds. Feeding HA to the broilers from 0 to 21 and from 0 to 42 d resulted only in 2.0 and 3.4 % increase, respectively. However, these improvements were not significant. Other scientists (Eren *et al.*, 2000) compared the effects of dietary HA (Farmagülatör DRY$^{TM}$) supplementation at 1.5 and 2.5 g kg$^{-1}$ on broiler performance from 0 to 42 d. Although there was no performance difference at 21 d, they found that dietary supplementation of humate at 2.5 g kg$^{-1}$ significantly improved the live weights of broilers at 42 d. Both references agreed that feeding HA to broilers had a growth-promoting effect only during the later stage of growth (22-42 d). Not only broilers but also pigs seem to exhibit an improvement in weight gain and feed efficiency during the late weaning period

and the late growing period due to addition of HA (Kim *et al.*, 2002). So our result supports these results due to the recovering tendency observed in body weight gain after two weeks of rearing. However, after 35 days, the groups with 2.4 and 4.8 g HA $kg^{-1}$ feed had a 4 % lower body mass than the control, and all HA groups did not reach the control. But feed intake was obviously not affected by HA, not attempt to compensate for the lower energy content of HA diets by higher consumption was made. But feed efficiency at 4.8 g HA $kg^{-1}$ feed was significantly inferior to the control.

A tendency to recover could also be seen in a company publication (Livestock R. Us., 2003) describing feed consumption of dairy cows dropped initially by 5 % and returned to normal at the end of the trial.

Some other literature is controversial to our findings and demonstrates many positive effects on growth performance. Ceylan and Ciftci, 2002, indicated the humic acid to be an alternative to antibiotic growth promoters in broiler diets by altering the microflora in the gastrointestinal system, eg., in the caecum (Shermer *et al.*, 1998). According to another companies report (Enviromate TM, 2002) the addition of humate substance to broiler ration should increase the yield mass on an average of 5 to 7%.

In summary results on HA in the literature are somewhat contradictory and not helpful in interpreting our data regarding growth, feed intake and feed efficiency. Any comparison as well as overall conclusions are additionally be complicated by the fact that the commercially available HA products differ in production (methodical procedure to separate HA form the original material and further refining) and the source and so biopotency. Further research should focus on the identification of optimal feed concentration and feeding strategy.

In our experiment the feed consumption was hampered during first two weeks of age in higher HA containing diets. But cumulative feed intake during first 4 week and during total duration of trial showed similar also repeated the situation of weight gain. The highest HA group (4.8 g $kg^{-1}$ feed) showed lower feed consumption during first two weeks of the trial.

The values for FCE showed that the 2.4 g and 4.8 g humic acid $kg^{-1}$ ration caused lower efficiency during initial two weeks of the trial. The situation also became slightly recovered after 4 weeks and at the end of the trial. However, the 4.8 g HA group was unable to recover and remained less efficient than control after 35 days. Related work also indicated insignificant changes in feed conversion efficiency due to continuous addition of humic acid (Eren *et al.*, 2000).

The slight and insignificant lower weight gain and feed utilisation of the HA groups could be discussed in connection with the not isocaloric and non isonitrogenous calculated diets which were offered during the trial. In formulating the diet, HA was exchanged against starch, the protein content may therefore not be influenced, but energy. However, by maximum inclusion of HA approximately 0.06 less MJ ME $kg^{-1}$ could be provided, an amount to low to be analytically confirmed and is considered negligible. It is therefore not assumed that

imbalances due to diet formulation are associated with negative effects or the lacking appearance of positive effects. On the other hand, binding capacities of HA are well known and experimentally demonstrated for some trace elements (Zn, Cu I) and for mycotoxins. The structure of HA also not excludes the potential for binding amino acids or small peptides. In a worst case assumption HA may reduce digestibility at all, and the recovery observed in the own experiment and by other authors could be regarded as a result of an oversupply of the older bird with nutrients compensating for lower digestibility.

Seffner, 1995, and 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. The measurements of weight and size of the thyroid gland done in our experiment did not reveal any differences due to the HA treatment. The dosages, 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 $kg^{-1}$. Our diet contained 2.5 mg added I $kg^{-1}$ feed. Both dosages were quite above the requirement or the allowance data. It is suggested, that (i) the high iodine of our broiler diet inhibited the potential occurrence of goitre in broiler, (ii) that our methods were to rough to detect a beginning iodine deficiency situation, and (iii) that the length of the broiler experiment was to short.

In our experiment the mortality of bird was within the accepted limit (Asghar *et al*, 2000, Naveed *et al.*, 1999) for the HA groups (1.25 to 5.0 %) but was higher (8.75 %) in the control group. The authors would accept mortality rates of 6.13 and 6.93 %, respectively. Our result could be considered as similar to the observation of Stepchenko *et al.*, 1991. They reported reduced unspecific deaths by 3 to 5% due to HA. The result is also supported by a company publication (HUMET, 2004) stating that two weeks oral administration of 10 mg humic acid $kg^{-1}$ bw could improve all parameters, which became pathologic after ischemic heart disease. But, other scientist (Kocabağli *et al.*, 2002) found no alteration of mortality in humic acid fed group. Also our data would despite the differences between control and all HA groups as mentioned above not seriously indicate an influence of HA on mortality, because (i) the low number of animals used in the experiment and (ii) the range of losses in the HA groups was from 1.25 % (1/80) to 5 % (4/80). Highest mortality was observed for 0.5 and 4.8 g HA $kg^{-1}$ feed, lowest figures for 0.6-2.4 g HA $kg^{-1}$. At least no dose inter-dependence could be observed.

However, under the controlled conditions of an experiment at laboratory scale, potential interactions of HA with commonly occurring pathogens or environmental stress can hardly be evaluated. But, many developing countries raise the broiler in open air house not environmentally controlled, therefore in some cases meat type breeds in a semi intensive system may tale profit from the inclusion HA, because humic acid may have the chance to protect the susceptible birds against pathogens, stress, polluted environment and other adverse effect mentioned earlier in the respective literature chapter 2.2.5. These suggestions also thought behind the findings of most positive effect studied in tropical countries, in most

cases the houses were not environmentally controlled. This could be the prerequisite of positive effects. So, the growth promoting and health protection effect combined with an improvement of gut health may become apparent when under sub-optimal poultry husbandry conditions health risk and stress factors can hardly be avoided. This idea is also supported by a study under unfavourable conditions (TeraVita ™, 2004) showing that the exchange of vitamin and antibiotics for sodium humate in the feed caused a decrease in the poultry losses for the first 40 days by 47 %. At the same time, their average weight gain increased by 10 %.

The data presented on the zootechnical effects of HA in chickens for fattening would not suffice to encourage producers or authorities for a use of HA as feed additive.

## 5.3 Coadministration of Semduramicin and Tiamulin

The interference study submitted is the first for semduramicin and the concurrent 5 days use of tiamulin. Also most studies on the compatibility of other ionophores with tiamulin are limited to a 3 days application of tiamulin.

### 5.3.1 Growth, Feed Consumption, Feed Efficiency and Water Intake

Severe clinical, often lethal, interactions between the ionophores monensin, salinomycin, maduramycin and narasin and the antibiotic tiamulin – both at routine dose levels - are described by several authors for chickens and turkeys, but also for pigs and rats (Hanrahan *et al.*, 1981; Weisman *et al.*, 1983; Umemura *et al.*, 1985; vanVleet *et al.*, 1987; Laczay *et al.,* 1989; Frigg *et al.,* 1983; Mazurkiewicz *et al.*, 1989a; Szucs *et al.*, 2000; Croubels *et al.,* 2001). The same toxic symptoms (loss of appetite, locomotor disturbances, ataxia, neurotoxic symptoms) were seen after administration of (an overdosage of) monensin alone or in combination with tiamulin (Hanrahan *et al.*, 1981; Umemura *et al.*, 1985; vanVleet *et al.*, 1987; Mazurkiewicz *et al.*, 1989a; Szucs *et al.*, 2000). Tiamulin gradually inhibits metabolism of ionophores in chicken organism, which in consequence leads to about the ionophore symptoms of poisoning (Mazurkiewicz *et al.*, 1989a; Umemura *et al.*, 1984; Meingassner *et al.*, 1979). These changes are, to a certain extent, due to marked disturbances in the transport of ions, i.e. sodium, potassium, calcium, magnesium, iron, zinc, copper, between myocytes and intercellular space (Mazurkiewicz *et al.*, 1989b). But actual mechanism of this toxic interaction is not known yet. First signs of intolerance are usually depressions in feed intake and weight gain. Therefore these most sensitive criteria are used in studying compatibility of tiamulin with other ionophores at routine use level or at lower dosages.

A transient depression of weight gain due to inclusion of tiamulin at 0.125 % in drinking water in broiler was recognized early of its history (Meingassner *et al.*, 1979). But the depression was insignificant and only numerical. In our experiment the same type of growth depression became evident showing no adverse effect in the tiamulin treated group except a temporary reduction during tiamulin

application. Lodge, 1988, could also demonstrate this type of a temporary reduction of water consumption when 250 mg tiamulin $L^{-1}$ water were administered in turkeys. Madej *et al.*, 1993, indicated that the administration of tiamulin (20 mg $kg^{-1}$ bw) caused depression of feed intake and lower body weight in all groups.

On the other hand tiamulin coadministration to the group continuously fed semduramicin showed strong adverse effect on water consumption, feed intake, and weight gain during administration. Many scientists attributed this type of adverse effects to the presence of tiamulin. Tiamulin administration between day 5 and 8 of the trial to birds treated with monensin-duokvin (duokvin, an antioxidant which alleviates severity of the interaction) caused significantly lower weight gain by 24.5% ($p<0.001$), and tiamulin was responsible for this reduction (Lehel *et al.*, 1995). But afterwards, in the observation period between day 9 and 12 that difference was no longer demonstrable, and chicks fed monensin-duokvin and tiamulin showed a 6.3% higher body mass gain, which compensated for the previous difference. Stipkovits *et al.*, 1992, found that co-administration of tiamulin (20 mg $kg^{-1}$ feed, lowered dosage!) and Salinomycin (60 mg $kg^{-1}$ feed) caused higher weight gain rather than salinomycin alone. Other scientists indicated that in broilers given 250 mg tiamulin $L^{-1}$ water on $18^{th}$ to $21^{st}$ day only numerical but not statistically significant differences in water consumption, feed consumption and weight gain occurred, but incompatibility with monensin, salinomycin and narasin was stated (Frigg *et al.*, 1983). The symptom recovered within two days after withdrawal. If no clinical signs become evident, as it was in our study, the slight interferences disappear and would later, after tiamulin withdrawal, be more or less fully compensated, dependent on the severity of interactions due to type and dosage of the ionophore coccidiostat and length of coadministration and the trial (the observation time) itself.

Similarly in our study the challenge of the metabolic system due to administration of tiamulin at $3^{rd}$ week caused reduced feed intake and resulted in lower weight gain. In isolated rat liver preparation, tiamulin was found to reduce the degradation and elimination of monensin (Meingassner *et al.*, 1979). The authors concluded that coadministration of these drugs causes an overdosage like condition in respect of monensin. Laczay *et al.*, 1990, demonstrated increased activities of some microsomal enzymes after simultaneous treatment of chickens with monensin and tiamulin, and came to a similar conclusion.

Our result that semduramicin alone had no effect on weight gain is supported by Pesti *et al.*, 1999c. They concluded that 25 mg $kg^{-1}$ feed in broilers from 18-35 day did not hamper weight gain, feed intake as well as feed conversion ratio when the protein level was optimum.

Pesti *et al.*, 2002, found that feed intake tended to be lower for semduramicin treatments with longer withdrawal periods ($P=0.191$ for 15 d and $P=0.057$ for 10 d). However, these same treatments were found to improve feed conversion ratios ($P=0.044$ for 15 d withdrawal and $P=0.013$ for 10 d withdrawal) from 0 to 49 d. Thus, semduramicin did not affect body gain, produced only subtle changes in feed intake, and significantly improved feed conversion ratio. This result was especially true for the longer withdrawal periods.

Fourteen birds (4.4 % mortality) died/were culled throughout the whole trial period. Reasons for culling were mainly "straddling" (osteochondrosis of the tibiotarsus of juvenile broiler chicks). Throughout the entire course of trial no therapeutic treatment was necessary. The reasons for mortality were mainly acute cardiovascular failure (myocarditis, fibrosis or degeneration of myocard) and disorders of the muscles or bones (myopathy, myositis, and osteochondrosis), both frequent causes for losses in commercial broiler fattening. The relatively high mortality rate (controlled institute conditions) might be in sum also the result of unfavourable temperature conditions. Almost the entire course of trial outdoor temperatures exceeded 30°C at noon. Also, in the trial house temperatures did not fall very often below 30°C, especially during tiamulin administration (outdoor temperatures of about 35°C), which apart from that depressed water intake.

From our data and in agreement with literature it is concluded that the depression of overall performance during co-administration of tiamulin and semduramicin is not a result of the single substances but of their interaction.

### 5.3.2 Blood Parameters after Compatibility Study

Results for enzyme activities seem to be somewhat inconsistent concerning trend and extent of the observed alterations. Moreover, mean values of the enzyme activities were accompanied by exceptionally high standard deviations or coefficients of variation, particularly for the creatine kinase with values between 0.32 and 0.63. In summary, the observed differences were not significant, and can obviously not be attributed to the treatment.

Level of glutathione, cytrochrome P-450 and glutathione peroxidase activity was significantly altered in tiamulin treated group within 2 hours of treatment (Mézes *et al.*, 1992). Lehel *et al.*, 1995, found that blood plasma AST activity of the chicks treated with tiamulin and monensin-duokvin was slightly increased (77±12 U $L^{-1}$) after the treatment period (on day 9), that increase was statistically significant ($p<0.05$). Increased AST activity was no longer demonstrable on day 13 and day 17. The values measured on days 1, 5, 9, 13, and 17 of the trial in chicks of other groups showed no major difference in blood plasma AST activity. As a result of the simultaneous administration of monensin-duokvin combination and tiamulin, blood plasma LDH activity was slightly elevated (58±9U $L^{-1}$) on days 9, 13, and 17 of the trial. However, that change was statistically not significant ($p>0.05$). Other antibiotics with this combinations as well as maduramicin and tiamulin caused no effect. All of these results indicate that changes in enzyme activities occur during treatment, and that elevated enzyme activities disappear after a certain withdrawal time. The observations are therefore in agreement with our results concerning insignificant alterations of enzyme and metabolite content because the blood was collected 17 days after withdrawal of tiamulin.

In experiments of Sakar *et al.*, 1991a, the enzyme (CK, ALD, LDH, AST, ALT and MDH) levels in plasma markedly jumped during simultaneous medication of narasin and tiamulin. However, two days after the discontinuation of both antibiotics from the food, the activity of all tested enzymes was on the average 5.5 times lower. Two weeks after the withdrawal of both from the food, complete recovery was

observed, and all tested enzymes were within normal range and well equalized. In a later study of the same group, Sakar *et al.*, 1991b, observed similar results with monensin and tiamulin, the authors confirmed the responsibility of tiamulin for temporary changes in of plasma constituents. Another study indicated no biochemical changes due to administration of tiamulin alone at 250 mg $L^{-1}$ water (Lodge *et al.*, 1988). But in our experiment tiamulin was withdrawn 17 days before blood sampling. Considering the withdrawal, tiamulin caused no changes in the enzymatic parameters. The changes occurring in the blood parameters due to the presence of semduramicin in the feed were also insignificant ($p>0.05$).

In our experiment no haematological changes were found, which was in agreement with the findings of Lodge *et al.*, 1988, in turkeys (250 mg tiamulin $L^{-1}$ water).

So, in conclusion co-administration of tiamulin with continuous supplementation of semduramicin would cause biochemical changes during that period but after 17 days withdrawal of tiamulin potential alterations of blood parameters disappeared. Also the haematocrit and electrolytes appear unchanged.

### 5.3.3 Clinical Effect due to Coadministration of Semduramicin and Tiamulin

In our experiment we did not find behavioural changes except reduced interest for water and feed intake during co-administration of tiamulin and semduramicin. But according to Mézes *et al.*, 1992, after oral administration of tiamulin and salinomycin, about one-third of the birds showed unsteady gait and signs of depression at 4 h after the treatment. The clinical signs subsided 8 h after the treatment and disappeared 24 h later. But this type of depression was not correlated with any morphological changes when post-mortem examination was conducted (Madej *et al.*, 1993) as well as during administration of tiamulin (20 mg $kg^{-1}$ bw). The authors also concluded that the tiamulin applied together with ionophoric anticoccidials to broiler chickens, might cause clinical symptoms of poisoning (monensin, narasin, salinomycin) according to dose as well as morphological changes in heart and two types (white and red) of skeletal muscle. Morphological changes after tiamulin and ionophoric anticoccidial administration are manifested by myo- and cardiomyopathies, and return to normal about $14^{th}$ day. Also our study revealed that the tiamulin caused no pathological changes after withdrawal 17 days before necropsy. On the other hand the continuation of semduramicin did not cause any changes in the pathological condition of birds at an age of 36 days.

Also, mortality, blood parameters and results of the histo-morphological examinations (day 36) of skeletal muscle, heart and nerve fibres did not give any evidence for a long-term effect of simultaneous administration of tiamulin and semduramicin. These findings are in good agreement with results of van Vleet and Ferrans (1984), who showed that large doses of monensin produce polyfocalmonophasic necrosis of skeletal muscle with type I fibre selectivity in swine, but regeneration developed early following injury and progresses rapidly to complete restoration of the necrotic muscles without residual fibrosis.

No adverse clinical signs were observed in any semduramicin medicated birds regardless of dose, nor did medication result in increased mortality (Elizabeth and Illyes, 1994). There were no haematological abnormalities, no gross pathological or histopathological abnormalities, and no adverse effects on litter.

### 5.3.4 Conclusions

Unanimous reports in the literature confirm the incompatibility of the ionophores monensin, maduramycin, salinomycin, and narasin with tiamulin at routine use levels. Semduramicin seems to be better tolerated as lasalocid (Comben, 1984; Lodge *et al.,* 1988). However, during coadministration, signs of interference between semduramicin and tiamulin become evident but disappear after a few days of tiamulin withdrawal. Two weeks thereafter the zootechnical as well as biochemical, haematological and morphological parameters confirm that any disorders have returned to normal physiological levels. That means for practical poultry operation, that eventually ill poultry on a semduramicin supplemented feed can be treated with tiamulin, it would be on the poultry manager to calculate economical costs of losses due to untreated illness against potential lower income due to lower poultry weight. The latter would only depend on time available in the remaining fattening period for compensation of lower weight gain due to reduced water and feed intake during the critical period of coadministration.

## 5.4 Carcass Characteristics in female Broilers fed Semduramicin for 5 Weeks followed by a Withdrawal Period of 5-8 Days.

The good growth which could be observed during the compatibility trial by supplementing feed with semduramicin continued at least for the selected birds, even after withdrawal of semduramicin caused. It can be stated that the withdrawal of the antibiotic semduramicin does not break growth rate as is it known from other (growth promoting) antibiotics (Gropp, 2003, personal communication) commonly caused by a sudden but transient decrease of feed intake.

Our finding is supported by the observations of Pesti *et al.*, 2002. The authors reared broilers for a period of 49 days under non-(coccidian) challenge conditions and obtained best performance with a 10 days withdrawal period.

Carcass characteristics remain unchanged by semduramicin according to statistical evaluation (except crude fat in breast muscle). But the semduramicin group showed after 5-8 days withdrawal partly better sensory characteristics than the control group. This finding could be traced back to a significantly increased fat content of breast muscle, however at a very low level (2.01 versus 1.57 %).

Semduramicin fulfils therefore the requirements of EU regulations (Regulation 1831/2003) that a feed additive should not affect animal produce (chemical, organoleptic and sensory) quality.

# 6. Summary

**Dose titration, tolerance and compatibility of some feed additives in broiler**

(93 pages, 17 figures, 38 tables, 25 tables in appendix and 234 references)

In two dose titration studies on chicken for fattening growth promoting efficacy and some aspects of safety of fumaric acid (FA), an approved preservative, and humic acid (HA), approved as veterinary drug, were examined. In a third experiment on chicken for fattening the compatibility of the ionophore coccidiostat semduramicin (AVIAX®), also approved at EU level, with the veterinary antibiotic tiamulin should be investigated.

In experiment 1 six isonitrogenous (22.8 % CP) and isocaloric diets (13 MJ ME $kg^{-1}$) containing 0, 1.25, 2.50, 3.75, 5.0, and 7.5 % FA, respectively, were fed for 26 days to 12 (replicates) x 8 (chicks per replicate) Lohmann-Hybrid newly hatched male chicks (48 g body weight). The diets consisted mainly of wheat and soybean meal. Mortality ranged between 0 and 4.2 %. Final body weight (feed efficiency) amounted to 1,506 (756), 1,597 (767), 1,532 (754), 1,485 (759), 1,342 (738), and 1,378 g (747 g gain $kg^{-1}$ feed) for the groups with 0, 1.25, 2.50, 3.75, 5.0, and 7.5 % FA, respectively. The 1.25 % FA group showed significantly ($p<0.05$) better weight gain than all other groups and better feed efficiency than the groups with 5.0 and 7.5 % FA. The higher gain was associated with higher feed intake. Body weight of the 5.0 and 7.5 % FA groups was significantly lower than that of the other groups. The relative weight of heart, liver and spleen was not affected by the treatment (except a lighter heart in the 1.25 % FA group). Pathological findings summarized to 5/10 in the 7.5 % FA group, to 1/10 in the control and to 2-3/10 in the other groups. The 7.5 % FA grouped showed also the highest incidence of kidney alterations. It is concluded that 1.25 % FA definitely promotes growth of broilers, that the margin of safety is about 3 (3.75/1.25).

In experiment 2 six diets containing 0, 0.3, 0.6, 1.2, 2.4, and 4.8 g Huminfeed® (74 % humic acid in DM) $kg^{-1}$, respectively, were fed for 35 days to 10 (replicates) x 8 (chicks per replicate) Lohmann-Hybrid newly hatched male chicks (46 g body weight). The diets consisted mainly of wheat and soybean meal (25.3 % CP, 12.8 MJ ME $kg^{-1}$). Mortality ranged between 1.25 and 8.75 %, being highest in the control group. Initially HA depressed weight gain, but recovery started in the third week, so that at the end no significant differences could be observed (body weight 2,408, 2,369, 2,335, 2,355, 2,310, and 2,301 g for the groups with 0, 0.3, 0.6, 1.2, 2.4, and 4.8 g Huminfeed® $kg^{-1}$). But body weight gain was negatively correlated with the HA content of the diets (y (weight gain) = 2327.98 – 0.0181x (Huminfeed content), r = -.280*). Feed intake was not affected by the treatment. Feed efficiency of the 4.8 g Huminfeed® $kg^{-1}$ group was significantly lower (689) than the control figure (723 g gain $kg^{-1}$ feed). The relative weight of liver, spleen, Bursa Fabricii and thyroid gland did not differ among the groups. Under laboratory conditions, HA did not show a growth promotion efficacy. The margin of safety is about 8 (2.4/0.3).

Experiment 3 (35 days) with a total of 320 female broilers chicks (day old Lohmann-Hybrid, 54 g) was conducted to study the effect of 25 mg semduramicin $kg^{-1}$ feed and the potential interference between semduramicin and tiamulin (administered from day 15 to 19 at a concentration of 250 mg $L^{-1}$ water) on zootechnical parameters (body weight, weight gain, feed intake, feed efficiency), blood parameters (electrolytes, enzymes; haematology) and health status (pathological examination). Group size was 10 replicates with 8 chicks each. A soybean meal, corn, soy oil diet was fed (24.4 % CP, 12.3 MJ ME $kg^{-1}$). Mortality ranged between 8.8 (control group) and 1.3 % (tiamulin group) and is seen in relation to high outside temperatures (>30°C).

All 4 groups grew equally during the first two weeks. Tiamulin medication from day 15 to 19 reduced significantly water intake, especially when concurrently given to birds fed semduramicin (control 1,117 g, tiamulin 957 g, semduramicin 1,164 g, semduramicin/tiamulin 809 g). Also feed intake was depressed in that period (control 1,424 g, tiamulin 415 g, semduramicin 418 g, semduramicin/tiamulin 376 g), however to a minor extent. As a consequence body weight gain of the semduramicin/tiamulin group in the third week was affected too (control 456 g, tiamulin 451 g, semduramicin 453 g, semduramicin/tiamulin 396 g). After 35 days, body weight (feed efficiency) was 2,062 (665), 2,067 (669), 2,084 (678), and 2,008 g (679 g gain $kg^{-1}$ feed) for the control, the tiamulin, the semduramicin and the semduramicin/tiamulin group, respectively. Only the feed intake of the semduramicin/tiamulin group was slightly but significantly lower, all other differences were not significant.

Ten birds per group were taken for blood samples and necropsy. The birds of the control group and of the semduramicin/tiamulin group were examined by histopathology. All data obtained did not give evidence for any adverse influence of the coadministration of tiamulin and semduramicin.

From the results it is concluded that semduramicin is only slightly incompatible with tiamulin. No clinical sings of incompatibility have been observed. The depression weight gain is more or less fully compensated during the following two weeks. Depending on the time available for a remaining fattening period, tiamulin can be administered under field conditions, if the broiler feed contains semduramicin.

At the end of the growth trial 20 broilers from each of the two feeding groups (with and without semduramicin) were transferred into floor pens for a 5 to 8 days withdrawal period. Five broilers from each feeding group were slaughtered at day 5, 6, 7 and 8. The semduramicin group had slightly higher body weight (2,569 vs. 2,477 g; $P>0.05$) at slaughter. Nevertheless, there were no treatment effects ($P>0.05$) on hot carcass weight, viscera (g or % of BW), dressing percentage, edible portions (breast muscles, haunch), fat and skin portions. Also the sensory characteristics (juiciness, tenderness, unpleasant pungent aroma, general impression) were not influenced by the treatment. However, the aroma/flavour of carcass in the semduramicin group was improved ($P<0.05$), probably due to a slightly higher intramuscularly fat content in meat ($P<0.05$).

# 7. Zusammenfassung

**Dosistitrations-, Toleranz- und Verträglichkeitsstudien mit ausgewählten Futterzusatzstoffen bei Broilern**

(93 Seiten, 17 Abbildungen, 38 Tabellen, 25 Anhangstabellen, 234 Literaturstellen)

Wachstumswirksamkeit und bestimmte Sicherheitsaspekte von Fumarsäure (FS), einem zugelassenen Konservierungsstoff, und Huminsäuren (HS), einem zugelassenen Veterinärtherapeutikum, sollten in zwei Dosistitrationsversuchen an Masthühnerküken untersucht werden. In einem dritten Versuch an Masthühnerküken sollte die Verträglichkeit des EU-weit zugelassenen Ionophor-Kokzidiostatikums Semduramicin (AVIAX®) mit dem Veterinär-Antibiotikum Tiamulin geprüft werden.

In Versuch 1 wurden sechs protein- (22,8 % RP) und energieäquivalente Rationen (13 MJ ME $kg^{-1}$) mit 0, 1,25, 2,50, 3,75, 5,00 bzw. 7,50 % FS über 26 Tage jeweils 96 (12 Wiederholungen x 8 Küken/Wiederholung) frisch geschlüpften Lohmann-Hybrid Hahnenküken (48 g Lebendmasse (LM)) angeboten. Die Rationen enthielten vorwiegend Weizen und Sojaextraktionsschrot. Die Mortalitätsrate bewegte sich zwischen 0 and 4,2 %. Die LM (Futterverwertung) bei Versuchsende betrug 1506 (756), 1597 (767), 1532 (754), 1485 (759), 1342 (738) bzw. 1378 g (747 g LM-Zunahme (LMZ) $kg^{-1}$ Futter) für die Gruppen mit 0, 1,25, 2,50, 3,75, 5,00 bzw. 7,50 % FS. Die LMZ der Gruppe mit 1,25 % FS war signifikant ($p<0.05$) höher als die aller anderen Gruppen, die Futterver-wertung besser als die der Gruppen mit 5,00 and 7,50 % FS. Die höhere LMZ ging mit einem gesteigerten Futterverzehr einher. Die LM der Gruppen mit 5,00 and 7,50 % FS war gegenüber den anderen Gruppen signifikant erniedrigt. Die relativen (auf die LM bezogenen) Massen von Herz, Leber und Milz blieben durch FS-Zulagen unbeeinflusst (mit Ausnahme einer geringeren Herzmasse der Gruppe mit 1,25 % FS). In der Gruppe mit 7,50 % FS wurden insgesamt 5/10 pathologische Befunde erhoben gegenüber nur 1/10 in der Kontrollgruppe und 2-3/10 in den anderen Versuchsgruppen. In dieser Gruppe wurde auch die höchste Anzahl an Nierenveränderungen registriert. Aus den Ergebnissen wird geschlossen, dass 1,25 % FS bei Broilern wachstumswirksam sind und dass die Sicherheitsspanne (höchste noch tolerierte Dosis im Verhältnis zur höchsten wirksamen Dosis) von FS bei etwa 3 (3,75/1,25) liegt.

In Versuch 2 wurden den Rationen 0, 0,3, 0,6, 1,2, 2,4 bzw. 4,8 g Huminfeed® (74 % Huminsäuren in der T) $kg^{-1}$ zugesetzt. Die Futtermischungen wurden jeweils 80 (10 Wiederholungen x 8 Küken/Wiederholung) frisch geschlüpften Lohmann-Hybrid Hahnen-küken (46 g LM) über 5 Wochen angeboten. Hauptbestandteile der Ration (25,3 % RP, 12,8 MJ ME $kg^{-1}$) waren Weizen und Sojaextraktionsschrot. Die Mortalitätsrate lag zwischen 1,25 und 8,75 % (höchster Wert in der Kontrollgruppe). HS führte in den ersten Wochen zu einer Verminderung der LMZ, eine Art Kompensation begann in der dritten Versuchswoche, so dass bei Versuchsende keine signifikanten Unterschiede zwischen den Gruppen mehr beobachtet werden konnten (LM 2408, 2369, 2335, 2355, 2310 bzw. 2301 g für die Gruppen mit 0, 0,3, 0,6, 1,2, 2,4 bzw. 4,8 g Huminfeed® $kg^{-1}$). Gleichwohl war

die LMZ mit dem Huminsäuregehalt des Futters negativ korreliert (y (LMZ) = 2327,98 – 0,0181x (Huminfeed-Zusatz), r = -.280*). Die Futteraufnahme blieb durch HS-Zusatz unbeeinflusst. Die Futterverwertung war durch den Zusatz von 4,8 g Huminfeed® $kg^{-1}$ (689) gegenüber dem Kontrollgruppenwert (723 g LMZ $kg^{-1}$ Futter) signifikant erniedrigt. Die relativen Massen von Leber, Milz, Bursa Fabricii und Schilddrüse wiesen keine Unterschiede zwischen den Gruppen auf. Unter den Bedingungen eines kontrollierten Institutsversuchs kann der HS daher keine Wachstumswirksamkeit zugesprochen werden. Der Sicherheitsbereich von Huminfeed beträgt etwa 8 (2,4/0,3).

In Versuch 3 (35 Tage) sollte an insgesamt 320 ein-Tag-alten Hennenküken (der Mastrasse Lohmann-Hybrid) die Auswirkung von Semduramicin (25 mg $kg^{-1}$ Futter) und einer möglichen Wechselwirkung von Semduramicin mit Tiamulin (15. bis 19. Tag, 250 mg $L^{-1}$ Wasser) auf die zootechnischen Parameter (LM, LMZ, Futterverwertung), Blutbestandteile (Elektrolyte, Enzyme, Hämatologie) und den Gesundheitsstatus (pathologische, makro- und mikroanatomische Untersuchung) geprüft werden. Die Gruppengröße betrug 10 Wiederholungen bei 8 Küken pro Wiederholung (56 g LM bei Versuchsbeginn). Die Sojaextraktionsschrot-Mais-Sojaöl-Ration enthielt 24,4 % RP bei 12,3 MJ ME $kg^{-1}$. Die Mortalitätsrate variierte zwischen 8,8 (Kontrollgruppe) und 1,3 % (Tiamulin-Gruppe) und muss unter Berücksichtigung der hohen Außentemperaturen bewertet werden (>30°C).

In den ersten beiden Versuchswochen wiesen alle Gruppen ein vergleichbares Wachstum auf. Tiamulin vom 15. bis 19. Tag reduzierte die Wasseraufnahme signifikant, besonders in der Semduramicin gefütterten Gruppe (Kontrolle 1117 g, Tiamulin 957 g, Semduramicin 1164 g, Semduramicin/Tiamulin 809 g). In diesem Abschnitt war auch der Futterverzehr verringert (Kontrolle 1424 g, Tiamulin 415 g, Semduramicin 418 g, Semduramicin/Tiamulin 376 g), allerdings in geringerem Ausmaß. Infolgedessen war auch die LMZ der Semduramicin/Tiamulin Gruppe in der dritten Woche beeinträchtigt (Kontrolle 456 g, Tiamulin 451 g, Semduramicin 453 g, Semduramicin/Tiamulin 396 g). Die LM (Futterverwertung) der Kontroll-gruppe, der Tiamulin-, Semduramicin- bzw. der Semduramicin/Tiamulin-Gruppe betrug bei Versuchsende 2062 (665), 2067 (669), 2084 (678) bzw. 2008 g (679 g LMZ $kg^{-1}$ Futter). Allein der Futterverzehr der Semduramicin/Tiamulin-Gruppe war geringfügig, gleichwohl signifikant vermindert. Alle anderen Unterschiede waren nicht signifikant.

Jeweils 10 Tieren einer Gruppe wurde Blut entnommen, dieselben Tiere wurden anschließend einer pathologisch-anatomischen Untersuchung unterzogen, die Kontroll- und die Semduramicin/Tiamulin-Tiere auch einer histopathologischen Untersuchung. Aus allen vorliegenden Daten konnte kein Hinweis auf eine poten-ziell abträgliche Wirkung der gleichzeitigen Verabreichung von Semduramicin und Tiamulin gewonnen werden.

Die Ergebnisse erlauben die Schlussfolgerung, dass Semduramicin eine nur geringe Unverträglichkeit mit Tiamulin aufweist. Klinische Anzeichen einer Inkom-patabilität traten nicht auf. Die während der gleichzeitigen Applikation von Semduramicin und Tiamulin aufgetretene Depression der LMZ wurde in den folgenden beiden Wochen mehr oder weniger vollständig kompensiert. Unter Feldbedingungen dürfte daher, abhängig von der Länge der noch verfügbaren

Mastperiode, einer Behandlung von Masthühnern, deren Futter Semduramicin enthält, mit Tiamulin nichts im Wege stehen.

Nach Beendigung des Wachstumsversuchs wurden jeweils 20 Broiler aus den Gruppen ohne und mit Semduramicin in Bodenhaltungseinheiten verbracht und für weitere 5-8 Tage mit der Kontrollmischung gefüttert (Absetzperiode). Am 5., 6., 7. und 8. Tag wurden jeweils 5 Tiere einer Rationsgruppe geschlachtet. Die Semduramicin-Tiere waren dabei etwas schwerer als die Kontrolltiere (2569 vs. 2,477 g; $p>0,05$). Veränderungen der Massen des Schlachtkörpers (warm), der Eingeweide (g oder % der Körpermasse), essbarer Anteile (Brustmuskel, Schenkel), von Fett und Haut sowie des Ausschlachtungsprozentsatzes durch Semduramicin traten nicht auf ($p>0,05$). Die sensorischen Charakteristika (Saftigkeit, Zartheit, stechendes Aroma, Gesamteindruck) blieben ebenfalls unbeeinflusst. Jedoch wurden Aroma/Geschmack der Semduramicin-Schlachtkörper besser ($p<0,05$) bewertet, möglicherweise aufgrund eines geringfügig, aber signifikant erhöhten intramuskulären Fettanteils im Fleisch dieser Tiere.

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*Appendix 1. Distribution of Cages in Poultry House for the Experiment 1*

| 1* | 2* | 3* | | 3 | 2 | 1 | | 1 | 2 | 3 | | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *62 (6)* | *67 (5)* | *72 (3)* | | *57 (2)* | *51 (4)* | *45 (1)* | | *27 (4)* | *33 (1)* | *39 (5)* | | *21 (6)* | *14 (3)* | *7 (2)* |
| *61 (2)* | *66 (1)* | *71 (4)* | | *56 (5)* | *50 (6)* | *44 (2)* | | *26 (5)* | *32 (6)* | *38 (2)* | | *20 (3)* | *13 (1)* | *6 (4)* |
| *60 (5)* | *65 (6)* | *70 (1)* | | *55 (1)* | *49 (2)* | *43 (3)* | | *25 (6)* | *31 (3)* | *37 (4)* | | *19 (2)* | *12 (5)* | *5 (1)* |
| *59 (3)* | *64 (4)* | *69 (2)* | | *54 (3)* | *48 (5)* | *42 (4)* | | *24 (1)* | *30 (2)* | *36 (3)* | | *18 (6)* | *11 (4)* | *4 (5)* |
| *58 (1)* | *63 (2)* | *68 (5)* | Floor | *53 (6)* | *47 (1)* | *41 (5)* | | *23 (3)* | *29 (4)* | *35 (6)* | Floor | *17 (1)* | *10 (2)* | *3 (6)* |
| | | | | *52 (4)* | *46 (3)* | *40 (6)* | | *22 (2)* | *28 (5)* | *34 (1)* | | *16 (5)* | *9 (3)* | *2 (4)* |
| E N T R Y | | | | | | | | | | | | *15 (4)* | *8 (6)* | *1 (3)* |

Size of each cage was 0.8 sq.m (0.8X1.0 m)
Position of cages as *1- Top; *2- Middle and *3- Lower.
Figure in parenthesis indicated the groups offering different amount of fumaric acid (FA) as below

(1)- Control;
(2)-1.25% FA;
(3)-2.5%FA;
(4)-3.75%FA;
(5)-5.0%FA;
(6)-7.50%FA

*Appendix 2. Distribution of Cages (used 60 out of 72) in Poultry House for Experiment 2*

| 1* | 2* | 3* | | 3 | 2 | 1 | | 1 | 2 | 3 | | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 62 (-) | 67 (-) | 72 (-) | | ***57 (4)*** | ***51 (5)*** | ***45 (1)*** | | ***27 (5)*** | ***33 (3)*** | ***39 (1)*** | | ***21 (2)*** | ***14 (4)*** | ***7 (6)*** |
| 61 (-) | 66 (-) | 71 (-) | | ***56 (3)*** | ***50 (2)*** | ***44 (6)*** | | ***26 (2)*** | ***32 (6)*** | ***38 (4)*** | | ***20 (3)*** | ***13 (1)*** | ***6 (5)*** |
| ***60 (3)*** | ***65 (5)*** | 70 (-) | | ***55 (1)*** | ***49 (4)*** | ***43 (5)*** | | ***25 (3)*** | ***31 (1)*** | ***37 (5)*** | | ***19 (6)*** | ***12 (2)*** | ***5 (4)*** |
| ***59 (1)*** | ***64 (2)*** | 69 (-) | | ***54 (6)*** | ***48 (3)*** | ***42 (4)*** | | ***24 (6)*** | ***30 (4)*** | ***36 (2)*** | | ***18 (1)*** | ***11 (5)*** | ***4 (3)*** |
| ***58 (6)*** | ***63 (4)*** | 68 (-) | Floor | ***53 (5)*** | ***47 (1)*** | ***41 (2)*** | | ***23 (1)*** | ***29 (5)*** | ***35 (3)*** | Floor | ***17 (4)*** | ***10 (6)*** | ***3 (2)*** |
| | | | | ***52 (2)*** | ***46 (6)*** | ***40 (3)*** | | ***22 (4)*** | ***28 (2)*** | ***34 (6)*** | | ***16 (5)*** | ***9 (3)*** | ***2 (1)*** |
| | | | | | | | | | | | | 15 (-) | 8 (-) | 1 (-) |

**ENTRY**

Size of each cage was 0.8 sq.m (0.8X1.0 m)
Position of cages as *1- Top; *2- Middle and *3- Lower.

Figure in parenthesis indicated the groups offering different amount (mg $kg^{-1}$ feed) of Humin Feed (HA) as below

(1) Control
(2) 300 mg
(3) 600 mg
(4) 1200 mg
(5) 2400 mg
(6) 4800 mg

*Appendix 3. Distribution of Cages (40 cages out of 72) in Poultry House for Experiment 3*

| 1* | 2* | 3* | | 3 | 2 | 1 | | 1 | 2 | 3 | | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 62 (-) | 67 (-) | 72 (-) | | 57 (-) | 51 (-) | 45 (-) | | 27 (-) | 33 (-) | 39 (-) | | 21 (-) | 14 (-) | 7 (-) |
| ***61 (4)*** | ***66 (3)*** | ***71 (2)*** | | 56 (-) | 50 (-) | 44 (-) | | 26 (-) | 32 (-) | 38 (-) | | 20 (-) | 13 (-) | 6 (-) |
| ***60 (3)*** | ***65 (2)*** | ***70 (1)*** | | 55 (-) | ***49 (1)*** | ***43 (2)*** | | ***25 (3)*** | ***31 (4)*** | 37 (-) | | ***19 (2)*** | ***12 (1)*** | ***5 (4)*** |
| ***59 (2)*** | ***64 (1)*** | ***69 (4)*** | | 54 (-) | ***48 (4)*** | ***42 (1)*** | | ***24 (2)*** | ***30 (3)*** | 36 (-) | | ***18 (1)*** | ***11 (4)*** | ***4 (3)*** |
| ***58 (1)*** | ***63 (4)*** | ***68 (3)*** | Floor | 53 (-) | ***47 (3)*** | ***41 (4)*** | | ***23 (1)*** | ***29 (2)*** | 35 (-) | Floor | ***17 (4)*** | ***10 (3)*** | ***3 (2)*** |
| | | | | 52 (-) | ***46 (2)*** | ***40 (3)*** | | ***22 (4)*** | ***28 (1)*** | 34 (-) | | ***16 (3)*** | ***9 (2)*** | ***2 (1)*** |
| | | | | | | | | | | | | 15 (-) | 8 (-) | 1 (-) |

**ENTRY**

Size of each cage was 0.8 sq.m (0.8X1.0 m)
Position of cages as *1- Top; *2- Middle and *3- Lower.
Figure in parenthesis indicated the group offering respective number of treatments as below
(1) Control
(2) Semduramicin feed (25 mg $kg^{-1}$)
(3) Control + 250mg Tiamulin $L^{-1}$ water ($15^{th}$ –$19^{th}$ day)
(4) Semduramicin feed (25 mg $kg^{-1}$) + 250mg Tiamulin $L^{-1}$ water ($15^{th}$ –$19^{th}$ day)

*Appendix 4. Composition of Vitamin Premix*
*(Addition in 2.5g of premix (equivalent to 1 kg complete feed)*

| Name of Vitamin | Amount | Name of Vitamin | Amount |
|---|---|---|---|
| [a]Vitamin A | 13,500 I.U | [b]Vitamin D3 | 1,500 I.U |
| a-DL-Tocopherol acetate | 50 mg | Menadione | 2 mg |
| Thiamine | 3 mg | Riboflovin | 5 mg |
| Pyridixine | 4 mg | Cobalamin | 15 µg |
| Biotin | 250 µg | Folic acid | 200 µg |
| Nicotenic acid | 60 mg | Calcium pantothenate | 30 µg |
| Choline (from choline chloride) | 750 mg | Ascorbic acid | 150 µg |

[a]Equivalent to 4.05 mg retinol $kg^{-1}$ [b]Equivalent to 37.5 µg cholecalciferol $kg^{-1}$

*Appendix 5. Composition of the trace element premix, Addition (mg) in 1.25 g of premix (equivalent to 1 kg complete feed)*

| Name of trace element | Amount | Name of trace element | Amount |
|---|---|---|---|
| Fe (from iron fumarate) | 125,00 | Zn (from zink sulphate) | 100,00 |
| Cu (from copper sulphate) | 10,00 | Mn (from manganese sulphate) | 50,00 |
| Se (from sodium selenite) | 0,25 | I (from potassium iodate) | 2,50 |
| Co (from cobalt sulphate) | 0,20 | | |

*Appendix 6. Fumaric Acid on Weight Gain (g/bird) of Broiler During 26 Days Trial*

| ***Code*** | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|
| *Fumaric acid (%)* | *0* | *1.25* | *2.5* | *3.75* | *5.0* | *7.5* |
| **Weight in different week** | | | | | | |
| Initial, | 47.6 | 47.6 | 47.6 | 47.6 | 47.6 | 47.7 |
| (±sd) | (0.2) | (0.1) | (0.1) | (0.1) | (0.1) | (0.1) |
| *Relative to (1)* | *100* | *100* | *100* | *100* | *100* | *100* |
| 1st | 194$^{ab}$ | 213$^{d}$ | 201$^{b}$ | 193$^{ab}$ | 181$^{c}$ | 188$^{ac}$ |
| (±sd) | (12) | (9) | (8) | (9) | (10) | (8) |
| *Relative to (1)* | *100* | *110* | *103* | *99* | *93* | *97* |
| 2nd | 550$^{a}$ | 587$^{c}$ | 558$^{a}$ | 537$^{a}$ | 482$^{b}$ | 503$^{b}$ |
| (±sd) | (30) | (18) | (20) | (21) | (30) | (21) |
| *Relative to (1)* | *100* | *107* | *101* | *98* | *88* | *92* |
| 3rd | 1068$^{a}$ | 1136$^{c}$ | 1081$^{a}$ | 1045$^{a}$ | 919$^{b}$ | 967$^{b}$ |
| (±sd) | (66) | (41) | (38) | (50) | (53) | (47) |
| *Relative to (1)* | *100* | *106* | *101* | *98* | *86* | *91* |
| 26th days | 1506$^{a}$ | 1597$^{c}$ | 1532$^{a}$ | 1485$^{a}$ | 1342$^{b}$ | 1378$^{b}$ |
| (±sd) | (83) | (49) | (48) | (48) | (65) | (49) |
| *Relative to (1)* | *100* | *106* | *102* | *99* | *89* | *92* |
| ***Weekly live weight gain*** | | | | | | |
| 1st | 147$^{ab}$ | 165$^{d}$ | 153$^{b}$ | 145$^{ab}$ | 133$^{c}$ | 140$^{ac}$ |
| (±sd) | (12) | (9) | (8) | (9) | (10) | (8) |
| *Relative to (1)* | *100* | *113* | *104* | *99* | *91* | *96* |
| 2nd | 356$^{ac}$ | 374$^{c}$ | 357$^{ac}$ | 344$^{a}$ | 300$^{b}$ | 315$^{b}$ |
| (±sd) | (25) | (14) | (15) | (17) | (22) | (15) |
| *Relative to (1)* | *100* | *105* | *100* | *96* | *85* | *89* |
| 3rd | 518$^{ac}$ | 549$^{c}$ | 523$^{ac}$ | 509$^{a}$ | 437$^{b}$ | 464$^{b}$ |
| (±sd) | (38) | (27) | (23) | (33) | (27) | (30) |
| *Relative to (1)* | *100* | *105* | *101* | *98* | *84* | *89* |
| 4th (5 days) | 438$^{abc}$ | 461$^{c}$ | 452$^{ac}$ | 440$^{ac}$ | 424$^{ab}$ | 411$^{b}$ |
| (±sd) | (29) | (24) | (18) | (19) | (25) | (35) |
| *Relative to (1)* | *100* | *106* | *104* | *101* | *98* | *95* |
| ***Cumulative live weight gain*** | | | | | | |
| 2 weeks | 503$^{a}$ | 539$^{c}$ | 510$^{a}$ | 489$^{a}$ | 434$^{b}$ | 456$^{b}$ |
| (±sd) | (30) | (18) | (20) | (21) | (30) | (21) |
| *Relative to (1)* | *100* | *107* | *102* | *97* | *86* | *91* |
| 3 weeks | 1021$^{a}$ | 1088$^{c}$ | 1033$^{a}$ | 998$^{a}$ | 871$^{b}$ | 919$^{b}$ |
| (±sd) | (66) | (41) | (38) | (50) | (52) | (47) |
| *Relative to (1)* | *100* | *107* | *101* | *98* | *85* | *90* |
| 4 weeks (26 days) | 1459$^{a}$ | 1549$^{c}$ | 1484$^{a}$ | 1438$^{a}$ | 1295$^{b}$ | 1331$^{b}$ |
| (±sd) | (82) | (49) | (48) | (48) | (65) | (49) |
| *Relative to (1)* | *100* | *106* | *102* | *99* | *89* | *91* |

$^{abc}$Similar superscripts in the same row differ significantly($p<0.05$); Absence of superscript indicated lack of significance differences ($p>0.05$)

*Appendix 7. Fumaric Acid on Feed Consumption (g/bird) of Broiler during 26 Days Trial*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|
| *Fumaric acid(%)* | *0* | *1.25* | *2.5* | *3.75* | *5.0* | *7.5* |
| **Per week** | | | | | | |
| 1st | $158^{a}$ | $172^{c}$ | $159^{a}$ | $154^{ab}$ | $146^{b}$ | $150^{ab}$ |
| (±sd) | (12) | (5) | (8) | (11) | (9) | (6) |
| *Relative to (1)* | *100* | *109* | *101* | *98* | *92* | *95* |
| $2^{nd}$ | $437^{ad}$ | $455^{d}$ | $439^{ad}$ | $417^{ab}$ | $383^{c}$ | $392^{bc}$ |
| (±sd) | (31) | (19) | (21) | (36) | (32) | (20) |
| *Relative to (1)* | *100* | *104* | *101* | *95* | *88* | *89* |
| $3^{rd}$ | $694^{a}$ | $714^{a}$ | $694^{a}$ | $674^{a}$ | $600^{b}$ | $627^{b}$ |
| (±sd) | (42) | (37) | (32) | (44) | (37) | (40) |
| *Relative to (1)* | *100* | *102* | *100* | *97* | *87* | *90* |
| $4^{th}$ (22-26days) | $641^{abc}$ | $681^{d}$ | $675^{cd}$ | $652^{acd}$ | $625^{ab}$ | $612^{b}$ |
| (±sd) | (39) | (27) | (26) | (34) | (31) | (33) |
| *Relative to (1)* | *100* | *106* | *105* | *101* | *97* | *95* |
| **Cumulative** | | | | | | |
| *1st* | $158^{a}$ | $172^{c}$ | $159^{a}$ | $154^{ab}$ | $146^{b}$ | $150^{ab}$ |
| (±sd) | (12) | (5) | (8) | (11) | (9) | (6) |
| *Relative to (1)* | *100* | *109* | *101* | *98* | *92* | *95* |
| $1^{st}$-$2^{nd}$ | $595^{ab}$ | $627^{a}$ | $599^{ab}$ | $572^{bc}$ | $529^{d}$ | $542^{cd}$ |
| (±sd) | (38) | (22) | (27) | (43) | (40) | (24) |
| *Relative to (1)* | *100* | *105* | *101* | *96* | *89* | *91* |
| $1^{st}$ -$3^{rd}$ | $1289^{ac}$ | $1341^{c}$ | $1293^{ac}$ | $1246^{a}$ | $1129^{b}$ | $1169^{b}$ |
| (±sd) | (77) | (57) | (55) | (78) | (67) | (58) |
| *Relative to (1)* | *100* | *103* | *100* | *96* | *88* | *91* |
| $1^{st}$ -$4^{th}$ (Day26) | $1930^{ac}$ | $2022^{c}$ | $1969^{ac}$ | $1898^{a}$ | $1754^{b}$ | $1781^{b}$ |
| (±sd) | (107) | (71) | (69) | (98) | (92) | (71) |
| *Relative to (1)* | *100* | *104* | *102* | *98* | *91* | *92* |

$^{abc}$Different superscript in the same row differ significantly($p<0.05$); Absence of superscript indicated lack of significance differences ($p>o.o5$)

*Appendix 8. Fumaric Acid on Feed Conversion Efficiency (g LWG kg$^{-1}$ FI) in Broiler during Different Period of the Trial*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|
| *Fumaric acid (%)* | *0* | *1.25* | *2.5* | *3.75* | *5.0* | *7.5* |
| **Per week** | | | | | | |
| 1st | 929$^{ab}$ | 960$^{b}$ | 961$^{b}$ | 943$^{ab}$ | 912$^{a}$ | 936$^{ab}$ |
| (±sd) | (50) | (49) | (34) | (34) | (33) | (39) |
| *Relative to (1)* | *100* | *103* | *103* | *101* | *98* | *101* |
| 2$^{nd}$ | 814$^{ab}$ | 823$^{b}$ | 814$^{ab}$ | 827$^{b}$ | 786$^{a}$ | 803$^{ab}$ |
| (±sd) | (14) | (19) | (18) | (53) | (18) | (12) |
| *Relative to (1)* | *100* | *101* | *100* | *101* | *97* | *99* |
| 3$^{rd}$ | 747$^{ab}$ | 769$^{c}$ | 752$^{ac}$ | 754$^{ac}$ | 728$^{b}$ | 739$^{ab}$ |
| (±sd) | (18) | (15) | (21) | (12) | (19) | (20) |
| *Relative to (1)* | *100* | *104* | *101* | *101* | *96* | *99* |
| 4$^{th}$ (22-26days) | 683 | 677 | 669 | 677 | 679 | 671 |
| (±sd) | (18) | (20) | (23) | (41) | (26) | (41) |
| *Relative to (1)* | *100* | *100* | *99* | *100* | *100* | *99* |
| **Cumulative** | | | | | | |
| *1$^{st}$* | 929$^{ab}$ | 960$^{a}$ | 961$^{a}$ | 943$^{ab}$ | 912$^{b}$ | 936$^{ab}$ |
| (±sd) | (50) | (49) | (34) | (34) | (33) | (39) |
| *Relative to (1)* | *100* | *103* | *103* | *101* | *98* | *101* |
| 1$^{st}$-2$^{nd}$ | 845$^{a}$ | 860$^{a}$ | 853$^{a}$ | 858$^{a}$ | 820$^{b}$ | 840$^{ab}$ |
| (±sd) | (16) | (14) | (14) | (46) | (14) | (11) |
| *Relative to (1)* | *100* | *102* | *101* | *102* | *97* | *99* |
| 1$^{st}$ -3$^{rd}$ | 792$^{a}$ | 812$^{c}$ | 799$^{ac}$ | 802$^{ac}$ | 771$^{b}$ | 786$^{a}$ |
| (±sd) | (14) | (29) | (15) | (22) | (11) | (13) |
| *Relative to (1)* | *100* | *103* | *101* | *101* | *97* | *99* |
| 1$^{st}$ -4$^{th}$ (Day26) | 756$^{ac}$ | 767$^{c}$ | 754$^{ac}$ | 759$^{ac}$ | 738$^{b}$ | 747$^{ab}$ |
| (±sd) | (10) | (9) | (16) | (21) | (17) | (16) |
| *Relative to (1)* | *100* | *102* | *100* | *101* | *98* | *99* |
| **Mortality** | **2.1** | **3.1** | **0** | **3.1** | **4.2** | **1.0** |

$^{abc}$Different superscripts in the same row differ significantly($p<0.05$); Absence of superscript indicated lack of significance differences ($p>o.o5$)

*Appendix 9. Live weight (g) of Humic Acid Fed Broilers in Different Weeks*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|
| *Humic acid (mg kg$^{-1}$ feed)* | *0* | *300* | *600* | *1200* | *2400* | *4800* |
| **Live weight** | | | | | | |
| Initial | 46.3 | 46.3 | 46.4 | 46.3 | 46.3 | 46.6 |
| (±sd) | (0.1) | (0.1) | (0.1) | (0.1) | (0.1) | (0.1) |
| *Relative to (1)* | *100* | *100* | *100* | *100* | *100* | *100* |
| 1st week | 193.4$^{a}$ | 193.5$^{a}$ | 192.0$^{a}$ | 192.3$^{a}$ | 185.0$^{a}$ | 174.2$^{b}$ |
| (±sd) | (8.9) | (5.6) | (7.7) | (4.7) | (7.5) | (6.1) |
| *Relative to (1)* | *100* | *100* | *99* | *99* | *96* | *90* |
| Birds out of trial | 1 | 0 | 0 | 0 | 0 | 0 |
| 2nd week | 547$^{a}$ | 541$^{a}$ | 543$^{a}$ | 536$^{a}$ | 521$^{a}$ | 483$^{b}$ |
| (±sd) | (19) | (14) | (20) | (18) | (35) | (24) |
| *Relative to (1)* | *100* | *99* | *99* | *98* | *95* | *88* |
| Birds out of trial | 1 | 0 | 0 | 2 | 1 | 0 |
| 3rd week | 1080$^{a}$ | 1072$^{a}$ | 1076$^{a}$ | 1074$^{a}$ | 1037$^{ab}$ | 991$^{b}$ |
| (±sd) | (36) | (47) | (42) | (31) | (70) | (43) |
| *Relative to (1)* | *100* | *99* | *100* | *99* | *96* | *92* |
| Birds out of trial | 4 | 2 | 0 | 2 | 1 | 0 |
| 4th week | 1723$^{a}$ | 1691$^{ab}$ | 1681$^{ab}$ | 1689$^{ab}$ | 1652$^{ab}$ (96) | 1622$^{b}$ |
| (±sd) | (60) | (92) | (60) | (61) | *96* | (66) |
| *Relative to (1)* | *100* | *98* | *98* | *98* | 1 | *94* |
| Birds out of trial | 4 | 4 | 0 | 2 | | 3 |
| 5th week | 2408 | 2369 | 2335 | 2355 | 2310 | 2301 |
| (±sd) | (60) | (107) | (115) | (115) | (114) | (109) |
| *Relative to (1)* | *100* | *98* | *97* | *98* | *96* | *96* |
| Birds out of trial | 7 | 4 | 1 | 2 | 1 | 4 |

$^{abc}$Different superscript in the same row differ significantly($p<0.05$)

*Appendix 10. Live Weight Gain (LWG) and Cumulative LWG per Bird (g) in Different Weeks*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|
| *Humic acid (mg kg$^{-1}$ feed)* | *0* | *300* | *600* | *1200* | *2400* | *4800* |
| **LWG** | | | | | | |
| 1st week | 147$^{a}$ | 147$^{a}$ | 146$^{a}$ | 146$^{a}$ | 139$^{a}$ | 129$^{b}$ |
| (±sd) | (8.8) | (5.6) | (7.8) | (4.8) | (7.5) | (6.1) |
| *Relative to (1)* | *100* | *100* | *99* | *99* | *94* | *87* |
| 2nd week | 353$^{a}$ | 348$^{a}$ | 351$^{a}$ | 344$^{a}$ | 336$^{a}$ | 309$^{b}$ |
| (±sd) | (12.5) | (13.0) | (13.5) | (14.6) | (28.8) | (19.1) |
| *Relative to (1)* | *100* | *98* | *99* | *97* | *95* | *88* |
| 3rd week | 533 | 531 | 533 | 538 | 515 | 507 |
| (±sd) | (28) | (36) | (30) | (23) | (37) | (21) |
| *Relative to (1)* | *100* | *99* | *100* | *101* | *97* | *95* |
| 4th week | 643 | 619 | 605 | 614 | 615 | 632 |
| (±sd) | (32) | (56) | (38) | (39) | (36) | (32) |
| *Relative to (1)* | *100* | *96* | *94* | *96* | *96* | *98* |
| 5th week | 685 | 679 | 654 | 666 | 658 | 679 |
| (±sd) | (21) | (36) | (70) | (82) | (51) | (55) |
| *Relative to (1)* | *100* | *99* | *96* | *97* | *96* | *99* |
| **Cumulative LWG** | | | | | | |
| 1st week | 147$^{a}$ | 147$^{a}$ | 146$^{a}$ | 146$^{a}$ | 139$^{a}$ | 129$^{b}$ |
| (±sd) | (8.8) | (5.6) | (7.8) | (4.8) | (7.5) | (6.1) |
| *Relative to (1)* | *100* | *100* | *99* | *99* | *94* | *87* |
| 2nd week | 500$^{a}$ | 495$^{a}$ | 497$^{a}$ | 490$^{a}$ | 475$^{a}$ | 437$^{b}$ |
| (±sd) | (19) | (14) | (20) | (17) | (35) | (24) |
| *Relative to (1)* | *100* | *99* | *99* | *98* | *95* | *87* |
| 3rd week | 1034$^{a}$ | 1026$^{a}$ | 1029$^{a}$ | 1028$^{a}$ | 990$^{ab}$ | 945$^{b}$ |
| (±sd) | (36) | (47) | (43) | (31) | (70) | (43) |
| *Relative to (1)* | *100* | *99* | *100* | *99* | *96* | *91* |
| 4th week | 1677$^{a}$ | 1645$^{ab}$ | 1634$^{ab}$ | 1642$^{ab}$ | 1606$^{ab}$ | 1576$^{b}$ |
| (±sd) | (60) | (92) | (61) | (61) | (96) | (66) |
| *Relative to (1)* | *100* | *98* | *97* | *98* | *96* | *94* |
| 5th week | 2361 | 2323 | 2289 | 2309 | 2263 | 2255 |
| (±sd) | (60) | (107) | (115) | (115) | (114) | (109) |
| *Relative to (1)* | *100* | *98* | *97* | *98* | *96* | *95* |

$^{abc}$Different superscript in the same row differ significantly ($p<0.05$)

*Appendix 11. Feed intake and cumulative feed intake per bird (gram) in different week*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|
| *Humic acid (mg kg$^{-1}$ feed)* | *0* | *300* | *600* | *1200* | *2400* | *4800* |
| Feed Intake | | | | | | |
| 0-7 days weight | 146$^{ab}$ | 149$^{a}$ | 144$^{ab}$ | 147$^{ab}$ | 151$^{a}$ | 138$^{b}$ |
| (±sd) | (9) | (6) | (11) | (6) | (8) | (5) |
| *Relative to (1)* | *100* | *102* | *99* | *101* | *104* | *95* |
| 8-14 days weight | 406$^{a}$ | 406$^{a}$ | 409$^{a}$ | 400$^{ab}$ | 399$^{ab}$ | 380$^{b}$ |
| (±sd) | (15) | (15) | (17) | (16) | (28) | (19) |
| *Relative to (1)* | *100* | *100* | *101* | *99* | *98* | *94* |
| 15-21 days weight | 670 | 682 | 674 | 691 | 679 | 676 |
| (±sd) | (19) | (38) | (34) | (25) | (47) | (23) |
| *Relative to (1)* | *100* | *102* | *101* | *103* | *101* | *101* |
| 22-28 days weight | 890 | 891 | 885 | 893 | 891 | 920 |
| (±sd) | (65) | (56) | (46) | (73) | (49) | (46) |
| *Relative to (1)* | *100* | *100* | *99* | *100* | *100* | *103* |
| 29-35 days weight | 1155 | 1136 | 1090 | 1124 | 1110 | 1158 |
| (±sd) | (57) | (37) | (59) | (59) | (67) | (58) |
| *Relative to (1)* | *100* | *98* | *94* | *97* | *96* | *100* |
| **Cumulative Feed Intake** | | | | | | |
| 0-7 days weight | 146$^{ab}$ | 149$^{a}$ | 144$^{ab}$ | 147$^{ab}$ | 151$^{a}$ | 138$^{b}$ |
| (±sd) | (9) | (6) | (11) | (6) | (8) | (5) |
| *Relative to (1)* | *100* | *102* | *99* | *101* | *104* | *95* |
| 0-14 days weight | 551$^{a}$ | 555$^{a}$ | 553$^{a}$ | 547$^{a}$ | 550$^{a}$ | 518$^{b}$ |
| (±sd) | (23) | (17) | (22) | (20) | (32) | (22) |
| *Relative to (1)* | *100* | *101* | *100* | *99* | *100* | *94* |
| 0-21 days weight | 1221 | 1237 | 1227 | 1238 | 1229 | 1194 |
| (±sd) | (36) | (55) | (50) | (39) | (75) | (42) |
| *Relative to (1)* | *100* | *101* | *100* | *101* | *101* | *98* |
| 0-28 days weight | 2111 | 2128 | 2112 | 2131 | 2120 | 2114 |
| (±sd) | (90) | (107) | (80) | (106) | (114) | (81) |
| *Relative to (1)* | *100* | *101* | *100* | *101* | *100* | *100* |
| 0-35 days weight | 3266 | 3264 | 3202 | 3256 | 3230 | 3271 |
| (±sd) | (122) | (121) | (131) | (132) | (162) | (123) |
| *Relative to (1)* | *100* | *100* | *98* | *100* | *99* | *100* |

$^{abc}$Different superscript in the same row differ significantly ($p<0.05$)

*Appendix 12. Weekly and Cumulative Feed Conversion Efficiency (g LWG/Kg FI) fed humic acid diet*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** | **(6)** |
|---|---|---|---|---|---|---|
| *Humic acid (mg kg$^{-1}$ feed)* | *0* | *300* | *600* | *1200* | *2400* | *4800* |
| Weekly FCE | | | | | | |
| 0-7 days weight | 1011$^{a}$ | 988$^{ab}$ | 1020$^{a}$ | 992$^{ab}$ | 921$^{c}$ | 930$^{bc}$ |
| (±sd) | (27) | (29) | (86) | (14) | (69) | (56) |
| *Relative to (1)* | *100* | *98* | *101* | *98* | *91* | *92* |
| 8-14 days weight | 871$^{a}$ | 857$^{ab}$ | 858$^{ab}$ | 859$^{ab}$ | 842$^{b}$ | 813$^{c}$ |
| (±sd) | (22) | (23) | (23) | (14) | (29) | (21) |
| *Relative to (1)* | *100* | *98* | *98* | *99* | *97* | *93* |
| 15-21 days weight | 796$^{a}$ | 778$^{ab}$ | 790$^{a}$ | 779$^{ab}$ | 759$^{bc}$ | 751$^{c}$ |
| (±sd) | (30) | (21) | (26) | (15) | (19) | (20) |
| *Relative to (1)* | *100* | *98* | *99* | *98* | *95* | *94* |
| 22-28 days weight | 724 | 694 | 683 | 690 | 692 | 687 |
| (±sd) | (40) | (45) | (23) | (42) | (38) | (26) |
| *Relative to (1)* | *100* | *96* | *94* | *95* | *96* | *95* |
| 29-35 days weight | 594 | 597 | 600 | 591 | 593 | 586 |
| (±sd) | (30) | (28) | (51) | (52) | (36) | (38) |
| *Relative to (1)* | *100* | *101* | *101* | *100* | 100 | *99* |
| **Cumulative FCE** | | | | | | |
| 0-7 days weight | 1011$^{a}$ | 988$^{ab}$ | 1020$^{a}$ | 992$^{ab}$ | 921$^{c}$ | 930$^{bc}$ |
| (±sd) | (27) | (29) | (86) | (14) | (69) | (56) |
| *Relative to (1)* | *100* | *98* | *101* | *98* | *91* | *92* |
| 0-14 days weight | 908$^{a}$ | 892$^{a}$ | 899$^{a}$ | 895$^{a}$ | 863$^{b}$ | 844$^{b}$ |
| (±sd) | (19) | (18) | (26) | (12) | (32) | (26) |
| *Relative to (1)* | *100* | *98* | *99* | *99* | *95* | *93* |
| 0-21 days weight | 847$^{a}$ | 829$^{a}$ | 839$^{a}$ | 830$^{a}$ | 805$^{b}$ | 791$^{b}$ |
| (±sd) | (23) | (17) | (14) | (11) | (22) | (20) |
| *Relative to (1)* | *100* | *98* | *99* | *98* | *95* | *93* |
| 0-28 days weight | 795$^{a}$ | 773$^{ab}$ | 774$^{ab}$ | 771$^{ab}$ | 758$^{bc}$ | 746$^{c}$ |
| (±sd) | (26) | (23) | (16) | (20) | (22) | (16) |
| *Relative to (1)* | *100* | *97* | *97* | *97* | *95* | *94* |
| 0-35 days weight | 723$^{a}$ | 712$^{ab}$ | 715$^{ab}$ | 709$^{ab}$ | 701$^{ab}$ | 689$^{b}$ |
| (±sd) | (22) | (22) | (22) | (26) | (21) | (22) |
| *Relative to (1)* | *100* | *98* | *99* | *98* | *97* | *95* |

$^{abc}$Different superscript in the same row differ significantly ($p<0.05$)

*Appendix 13. Live Weight (g/bird/week) During Compatibility Study of Tiamulin and Semduramicin on Growth Performance of Broiler*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| *Semduramicin (mg $kg^{-1}$ feed)* | *None* | *None* | *25* | *25* |
| *Tiamulin (mg $L^{-1}$ water) (15-19th days)* | *None* | *250* | *None* | *250* |
| Initial, (±sd) | 54 (0.2) | 54 (0.2) | 54 (0.5) | 54(0.1) |
| *Relative to (1)* | *100* | *100* | *100* | *100* |
| 7th, (±sd) | 216 (4) | 213 (6) | 214 (9) | 211 (5) |
| *Relative to (1)* | *100* | 99 | 99 | 98 |
| *Birds out of trial | 0 | 0 | 0 | 0 |
| 14th, (±sd) | 547 (17) | 545 (16) | 542 (18) | 540 (16) |
| *Relative to (1)* | *100* | *100* | *99* | *99* |
| Birds out of trial | 2 | 0 | 0 | 0 |
| 21st, (±sd) | $1003^{a}$ (27) | $996^{a}$ (29) | $994^{a}$ (26) | $936^{b}$ (31) |
| *Relative to (1)* | *100* | *99* | *99* | *93* |
| Birds out of trial | 5 | 1 | 3 | 0 |
| 28th, (±sd) | $1497^{ab}$ (59) | $1520^{a}$ (47) | $1527^{a}$ (47) | $1458^{b}$ (56) |
| *Relative to (1)* | *100* | *102* | *102* | *97* |
| Birds out of trial | 7 | 1 | 4 | 1 |
| 35th, (±sd) | 2062 (89) | 2067 (72) | 2084 (58) | 2008 (70) |
| *Relative to (1)* | *100* | *100* | *101* | *97* |
| Birds out of trial | 7 | 1 | 4 | 2 |
| Cage weight, (±sd) | 15083 (2101) | 16347 (1101) | 15828 (980) | 15669(1087) |
| *Relative to (1)* | *100* | *108* | *105* | *104* |

*Cumulative total number of birds per treatment died or removed from the cages

$^{abc}$Different superscript in the same row differ significantly ($p<0.05$); Absence of superscript indicated lack of significance differences ($p>0.05$).

*Appendix 14. Live Weight Gain (g/bird/week) During Compatibility Study of Tiamulin and Semduramicin on the Growth Performance of Broiler*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| *Semduramicin (mg $kg^{-1}$ feed)* | *None* | *None* | *25* | *25* |
| *Tiamulin (mg $L^{-1}$ water) (15-19th days)* | *None* | *250* | *None* | *250* |
| **Gain in week** | | | | |
| 1st week, (±sd) | 161 (4) | 159 (6) | 160 (9) | 157 (5) |
| *Relative to (1)* | *100* | *98* | *99* | *97* |
| 2nd week, (±sd) | 331 (16) | 332 (11) | 328 (11) | 329 (12) |
| *Relative to (1)* | *100* | *100* | *99* | *99* |
| 3rd week, (±sd) | $456^{a}$ (14) | $451^{a}$ (18) | $453^{a}$(24) | $396^{b}$ (24) |
| *Relative to (1)* | *100* | *99* | *99* | *87* |
| 4th week, (±sd) | 494 (52) | 524 (28) | 533 (28) | 522 (34) |
| *Relative to (1)* | *100* | *106* | *108* | *106* |
| 5th week, (±sd) | 564 (33) | 547 (37) | 557 (28) | 550 (31) |
| *Relative to (1)* | *100* | *97* | *99* | *97* |
| **Cumulative gain** | | | | |
| Initial to 2nd, (±sd) | 493 (17) | 490 (16) | 488 (18) | 486 (16) |
| *Relative to (1)* | *100* | *100* | *99* | *99* |
| 2nd to 4th, (±sd) | $950^{ab}$ (54) | $976^{a}$ (38) | $985^{a}$ (39) | $918^{b}$ (46) |
| *Relative to (1)* | *100* | *103* | *104* | *97* |
| 3rd and 4th, (±sd) | 494 (52) | 524 (28) | 533 (28) | 522 (34) |
| *Relative to (1)* | *100* | *106* | *108* | *106* |
| Initial to 4th, (±sd) | $1443^{ab}$ (59) | $1466^{a}$ (47) | $1473^{a}$ (47) | $1404^{b}$ (56) |
| *Relative to (1)* | *100* | *102* | *102* | *97* |
| 3rd to 5th, (±sd) | 1059 (80) | 1071 (48) | 1090 (39) | 1072 (55) |
| *Relative to (1)* | *100* | *101* | *103* | *101* |
| Initial to 5th, (±sd) | 2008 (89) | 2013 (72) | 2030 (58) | 1954 (70) |
| *Relative to (1)* | *100* | *100* | *101* | *97* |

$^{abc}$Different superscripts in the same row differ significantly($p<0.05$); Absence of superscript indicated lack of significance differences ($p>o.o5$)

*Appendix 15. Feed Consumption (g/bird/week) During Compatibility Study of Tiamulin and Semduramicin on Growth Performance of Broiler*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| *Semduramicin (mg $kg^{-1}$ Feed)* | *None* | *None* | *25* | *25* |
| *Tiamulin (mg $L^{-1}$ water) (15-19th days)* | *None* | *250* | *None* | *250* |
| **Weeks** | | | | |
| 1st (±sd) | 174 (5) | 168 (9) | 168 (10) | 168 (8) |
| *Relative to (1)* | *100* | *97* | *97* | *97* |
| 2nd (±sd) | 398 (15) | 398 (10) | 398 (13) | 391 (11) |
| *Relative to (1)* | *100* | *100* | *100* | *98* |
| 3rd (±sd) | $617^{a}$ (24) | $597^{a}$ (20) | $607^{a}$ (24) | $558^{b}$ (20) |
| *Relative to (1)* | *100* | *97* | *98* | *90* |
| 4th (±sd) | $806^{ab}$(38) | $832^{a}$ (56) | $817^{ab}$(35) | $780^{b}$ (37) |
| *Relative to (1)* | *100* | *103* | *101* | *97* |
| 5th (±sd) | 1023 (38) | 1013 (49) | 1006 (67) | 981 (48) |
| *Relative to (1)* | *100* | *99* | *98* | *96* |
| **Cumulative** | | | | |
| 1st and 2nd (±sd) | 572 (18) | 567 (16) | 566 (12) | 559 (15) |
| *Relative to (1)* | *100* | *99* | *99* | *98* |
| 3rd and 4th (±sd) | $1423^{a}$ (51) | $1430^{a}$ (71) | $1424^{a}$ (56) | $1337^{b}$ (52) |
| *Relative to (1)* | *100* | *100* | *100* | *94* |
| 1st to 4th (±sd) | $1995^{a}$ (63) | $1996^{a}$ (78) | $1990^{a}$ (57) | $1897^{b}$ (57) |
| *Relative to (1)* | *100* | *100* | *100* | *95* |
| 4th and 5th, (±sd) | 1829 (69) | 1846 (92) | 1822 (94) | 1761 (80) |
| *Relative to (1)* | *100* | *101* | *100* | *96* |
| 1st to 5th, (±sd) | $3018^{a}$ (69) | $3009^{a}$ (115) | $2996^{a}$ (117) | $2877^{b}$ (93) |
| *Relative to (1)* | *100* | *100* | *99* | *95* |

$^{abc}$Different superscripts in the same row differ significantly($p<0.05$); Absence of superscript indicated lack of significance differences($p>0.05$).

*Appendix 16. Weekly Feed Conversion Efficiency (FCE; gLWG/kg feed intake/bird) and Feed Conversion Ratio (FCR; kg feed/kg gain) During Compatibility Study of Tiamulin and Semduramicin on the Growth Performance of Broiler*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| *Semduramicin (mg $kg^{-1}$ feed)* | *None* | *None* | *25* | *25* |
| *Tiamulin (mg $L^{-1}$ water) (15-19th days)* | *None* | *250* | *None* | *250* |
| **FCE** | | | | |
| $1^{st}$, (±sd) | 929 (32) | 944 (42) | 952 (39) | 936 (48) |
| *Relative to (1)* | *100* | *102* | *102* | *101* |
| $2^{nd}$,(±sd) | 833 (20) | 833 (17) | 825 (35) | 824 (13) |
| *Relative to (1)* | *100* | *100* | *99* | *101* |
| $3^{rd}$ (±sd) | $740^{a}$ (20) | $756^{a}$ (24) | $746^{a}$ (35) | $710^{b}$ (29) |
| *Relative to (1)* | *100* | *102* | *101* | *96* |
| $4^{th}$ (±sd) | $613^{a}$ (51) | $631^{ab}$ (32) | $652^{ab}$ (28) | $668^{b}$ (22) |
| *Relative to (1)* | *100* | *103* | *106* | *109* |
| $5^{th}$ (±sd) | 552 (25) | 540 (27) | 555 (34) | 561 (23) |
| *Relative to (1)* | *100* | *98* | *101* | *102* |
| **FCE(Cumulative)** | | | | |
| 1st and $2^{nd}$ (±sd) | 862 (20) | 866 (18) | 862 (29) | 870 (19) |
| *Relative to (1)* | *100* | *100* | *100* | *101* |
| 3rd and $4^{th}$ (±sd) | 668 (27) | 683 (27) | 692 (23) | 686 (20) |
| *Relative to (1)* | *100* | *102* | *104* | *103* |
| 1st to $4^{th}$ (±sd) | 724 (18) | 735 (24) | 740 (16) | 740 (16) |
| *Relative to (1)* | *100* | *102* | *102* | *102* |
| 4th and $5^{th}$ (±sd) | 579 (34) | 581 (24) | 599 (27) | 609 (19) |
| *Relative to (1)* | *100* | *100* | *104* | *105* |
| 1st to $5^{th}$ (±sd) | 665 (20) | 669 (22) | 678 (19) | 679 (16) |
| *Relative to (1)* | *100* | *101* | *102* | *102* |
| **FCR (Cumulative)** | | | | |
| Initial to $4^{th}$ (±sd) | 1.38 (0.04) | 1.36(0.05) | 1.35(0.03) | 1.35 (0.03) |
| *Relative to (1)* | *100* | *98* | *98* | *98* |
| Initial to $5^{th}$ (±sd) | 1.59 (0.05) | 1.5 (0.05) | 1.48(0.04) | 1.47 (0.03) |
| *Relative to (1)* | *100* | *101* | *98* | *98* |

$^{abc}$Values with different superscripts in the same row are different(P<0.05); Absence of superscript indicates lack of significance differences(p>o.o5)

*Appendix 17. Daily Water Consumption (g/bird) in Third Week During Tiamulin Supplementation to Know the Compatibility Study With Semduramicin in Growth Performance of Broiler*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| *Semduramicin (mg kg$^{-1}$ feed)* | *None* | *None* | *25* | *25* |
| *Tiamulin (mg L$^{-1}$ water) (15-19th days)* | *None* | *250* | *None* | *250* |
| **Daily** | | | | |
| 15$^{th}$ (±sd) | 175$^{a}$ (19) | 137$^{b}$ (30) | 178$^{a}$ (22) | 112$^{b}$ (28) |
| *Relative to (1)* | *100* | *78* | *101* | *64* |
| 16$^{th}$ (±sd) | 194$^{a}$ (37) | 172$^{ab}$ (8) | 219$^{c}$ (21) | 150$^{b}$ (14) |
| *Relative to (1)* | *100* | *88* | *113* | *77* |
| 17$^{th}$ (±sd) | 237$^{a}$ (15) | 202$^{b}$ (11) | 243$^{a}$ (20) | 173$^{c}$ (15) |
| *Relative to (1)* | *100* | *86* | *103* | *73* |
| 18$^{th}$ (±sd) | 245$^{a}$ (22) | 212$^{b}$ (9) | 252$^{a}$ (19) | 184$^{c}$ (14) |
| *Relative to (1)* | *100* | *87* | *103* | *75* |
| 19$^{th}$ (±sd) | 266$^{a}$ (19) | 234$^{b}$ (14) | 272$^{a}$ (25) | 190$^{c}$ (17) |
| *Relative to (1)* | *100* | *89* | *102* | *72* |
| 20th, (±sd) | 259$^{a}$ (20) | 244$^{ab}$ (15) | 268$^{a}$ (30) | 228$^{b}$ (20) |
| *Relative to (1)* | *100* | *94* | *103* | *88* |
| 21st, (±sd) | 289$^{a}$ (21) | 286$^{a}$ (20) | 290$^{a}$ (21) | 260$^{b}$ (21) |
| *Relative to (1)* | *100* | *99* | *100* | *90* |
| **Cumulative** | | | | |
| 15th to 19$^{th}$ (±sd) | 1117$^{a}$ (59) | 957$^{b}$ (52) | 1164$^{a}$ (97) | 809$^{c}$ (62) |
| *Relative to (1)* | *100* | *86* | *104* | *72* |
| 15th to 21$^{st}$ (±sd) | 1665$^{a}$ (93) | 1486$^{b}$ (71) | 1721$^{a}$ (138) | 1296$^{c}$ (96) |
| *Relative to (1)* | *100* | *89* | *103* | *78* |

$^{ab}$Values with different superscripts in the same row are different($P<0.05$); Absence of superscript indicated lack of significance differences($p>o.o5$)

*Appendix 18. Daily Feed Intake (g/bird) in Third Week During Tiamulin Supplementation to Know the Compatibility with Semduramicin in Growth Performance of Broiler*

| **Code** | **(1)** | **(2)** | **(3)** | **(4)** |
|---|---|---|---|---|
| *Semduramicin (mg kg$^{-1}$ feed)* | *None* | *None* | *25* | *25* |
| *Tiamulin (mg L$^{-1}$ water) (15-19th days)* | *None* | *250* | *None* | *250* |
| **Daily** | | | | |
| 15$^{th}$ (±sd) | 84$^{a}$ (4) | 80$^{a}$ (7) | 82$^{a}$ (3) | 73$^{b}$ (7) |
| *Relative to (1)* | *100* | *96* | *97* | *87* |
| 16$^{th}$ (±sd) | 68$^{a}$ (10) | 69$^{a}$ (3) | 70$^{a}$ (4) | 63$^{b}$ (5) |
| *Relative to (1)* | *100* | *102* | *103* | *93* |
| 17$^{th}$ (±sd) | 85$^{a}$ (5) | 83$^{a}$ (5) | 84$^{a}$ (4) | 75$^{b}$ (5) |
| *Relative to (1)* | *100* | *97* | *99* | *88* |
| 18$^{th}$ (±sd) | 84$^{a}$ (4) | 83$^{a}$ (3) | 84$^{a}$ (3) | 78$^{b}$ (5) |
| *Relative to (1)* | *100* | *98* | *99* | *93* |
| 19$^{th}$ (±sd) | 102$^{a}$ (6) | 100$^{a}$ (10) | 99$^{a}$ (9) | 86$^{b}$ (4) |
| *Relative to (1)* | *100* | *98* | *97* | *84* |
| 20$^{th}$ (±sd) | 96$^{a}$ (6) | 87$^{b}$ (6) | 93$^{ab}$ (7) | 95$^{a}$ (6) |
| *Relative to (1)* | *100* | *91* | *97* | *99* |
| 21$^{st}$ (±sd) | 98$^{a}$ (10) | 95$^{a}$ (7) | 96$^{a}$ (8) | 86$^{b}$ (4) |
| *Relative to (1)* | *100* | *97* | *98* | *88* |
| **Cumulative** | | | | |
| 15th to 19$^{th}$ (±sd) | 424$^{a}$ (14) | 415$^{a}$ (16) | 418$^{a}$ (15) | 376$^{b}$ (15) |
| *Relative to (1)* | *100* | *98* | *99* | *89* |
| 15th to 21$^{st}$ (±sd) | 617$^{a}$ (24) | 597$^{a}$ (20) | 607$^{a}$ (24) | 558$^{b}$ (20) |
| *Relative to (1)* | *100* | *97* | *98* | *90* |

$^{ab}$Values with different superscripts in the same row are different($P<0.05$); Absence of superscript indicated lack of significance differences($p>0.05$)

*Appendix 19. Scheme From Cages into Floor Pens and Individual Body Weight (day 35) of Broiler Chicks*

| **Code** | **(1)** | | | **Code** | **(3)** | | |
|---|---|---|---|---|---|---|---|
| *Cage no* | *BW (g/bird)* | *Pen No* | *BW (g/bird/pen)* | *Cage no* | *BW (g/bird)* | *Pen No* | *BW (g/bird/pen)* |
| 2 | 2250 | 1 | | 3 | 2143 | 24 | |
| 2 | 2040 | 1 | | 3 | 2067 | 24 | |
| 12 | 2155 | 1 | | 9 | 2240 | 24 | |
| 12 | 1958 | 1 | 2101 | 9 | 2180 | 24 | 2158 |
| 18 | 2201 | 2 | | 19 | 2114 | 23 | |
| 18 | 2180 | 2 | | 19 | 2180 | 23 | |
| 23 | 1975 | 2 | | 24 | 2070 | 23 | |
| 23 | 2030 | 2 | 2097 | 24 | 2106 | 23 | 2118 |
| 28 | 2132 | 3 | | 29 | 2052 | 22 | |
| 28 | 1975 | 3 | | 29 | 2128 | 22 | |
| 42 | 2070 | 3 | | 43 | 2087 | 22 | |
| 42 | 1941 | 3 | 2030 | 43 | 2030 | 22 | 2074 |
| 49 | 2250 | 4 | | 46 | 2102 | 21 | |
| 49 | 2006 | 4 | | 46 | 2074 | 21 | |
| 58 | 2220 | 4 | | 59 | 2023 | 21 | |
| 58 | 2064 | 4 | 2135 | 59 | 2208 | 21 | 2102 |
| 64 | 2081 | 5 | | 65 | 2209 | 20 | |
| 64 | 1983 | 5 | | 65 | 2099 | 20 | |
| 70 | 2262 | 5 | | 71 | 2090 | 20 | |
| 70 | 1936 | 5 | 2066 | 71 | 2071 | 20 | 2117 |
| Mean weight, | | | 2085 | Mean weight, | | | 2114 |
| *(±sd)* | | | *(112)* | *(±sd)* | | | *(62)* |
| *Relative to (1)* | | | *100* | *Relative to (1)* | | | *101* |
| 35th day | | | 2062 | 35th day | | | 2084 |
| *(±sd)* | | | *(89)* | *(±sd)* | | | *(58)* |
| *Relative to (1)* | | | *100* | *Relative to (1)* | | | *101* |

*Appendix 20. Performance Data of the Control and the Semduramicin Group Over 35 Days (battery)*

| **Code** | **(1)** | **(3)** |
|---|---|---|
| *Semduramycin (mg kg$^{-1}$ feed),Day 1-35* | ----- | *25* |
| Initial body weight, (±sd) | 54.0 (0.2) | 54.0 (0.5) |
| *Relative to (1)* | *100* | *100* |
| Body weight at 35 days, (±sd) | 2062 (89) | 2084 (58) |
| *Relative to (1)* | *100* | *101* |
| Total cage weight, (±sd) | 15083 (2101) | 15828 (980) |
| *Relative to (1)* | *100* | *105* |
| Weight gain, (±sd) | 2008 (89) | 2030 (58) |
| *Relative to (1)* | *100* | *101* |
| Feed intake, (±sd) | 3018 (69) | 2996 (117) |
| *Relative to (1)* | *100* | *99* |
| Feed efficiency, (±sd) | 665 (20) | 678 (19) |
| *Relative to (1)* | *100* | *102* |
| Feed conversion, (±sd) | 1.59 (0.05) | 1.48 (0.04) |
| *Relative to (1)* | *100* | *98* |
| Birds died (% of total birds in treatment) | 7 (8.8) | 4 (5.0) |
| *Without semduramicin, Day 36-43* | *(1)* | *(3)* |
| LW (selected birds at day 35), (±sd) | 2085 (112) | 2114 (62) |
| *Relative to (1)* | *100* | *101* |
| LW (during slaughter), (±sd) | 2477 (178) | 2569 (168) |
| *Relative to (1)* | *100* | *104* |
| LWG (day 35 to slaughter), (±sd) | 60 (9) | 70 (10) |
| *Relative to (1)* | *100* | *116* |

All the parameters showed insignificant differences at 5 % level

*Appendix 21. Weight(g) of Slaughtered Bird and their Different Parts After Overnight Cooling ($4^0$C)*

| ***Code*** | | **(1)** | **(3)** | **(1)** | **(3)** |
|---|---|---|---|---|---|
| *Semduramycin* | *Day 1-35* | ----- | *25* | ----- | *25* |
| *(mg $kg^{-1}$ feed),* | *Day 36-43* | ----- | ---- | ----- | ---- |
| | | ***Gram*** | | ***% of Slaughter weight*** | |
| LW (during slaughter) | | 2477 | 2569 | - | - |
| (±sd) | | (178) | (168) | | |
| *Relative to (1)* | | *100* | *104* | | |
| #Carcass (Incl. giblets) | | 1933.8 | 2014.5 | 78.0 | 78.4 |
| (±sd) | | (166) | (157) | (2.3) | (1.9) |
| *Relative to (1)* | | *100* | *104* | *100* | *100* |
| #Carcass | | 1848.0 | 1931.0 | 74.6 | 75.1 |
| (±sd) | | (162) | (152) | (2.4) | (2.0) |
| *Relative to (1)* | | *100* | *104* | *100* | *101* |
| #Carcass, halfs | | 1814.5 | 1896.7 | - | - |
| (±sd) | | (158) | (150) | | |
| *Relative to (1)* | | *100* | *105* | | |
| Shanks | | 82.1 | 84.3 | 3.32 | 3.29 |
| (±sd) | | (8.6) | (4.5) | (0.28) | (0.22) |
| *Relative to (1)* | | *100* | *103* | *100* | *99* |
| Liver (without gallbladder) | | 55.0 | 53.4 | 2.22 | 2.07 |
| (±sd) | | (9.8) | (8.1) | (0.36) | (0.25) |
| *Relative to (1)* | | *100* | *97* | *100* | *93* |
| Heart | | 10.3 | 10.8 | 0.42 | 0.42 |
| (±sd) | | (1.4) | (0.9) | (0.05) | (0.04) |
| *Relative to (1)* | | *100* | *104* | *100* | *100* |
| Gizzard | | 20.4 | 19.4 | $0.83^a$ | $0.76^b$ |
| (±sd) | | (3.0) | (2.5) | (0.1) | (0.1) |
| *Relative to (1)* | | *100* | *95* | *100* | ***91*** |
| Abdominal fat | | 30.4 | 30.4 | 1.23 | 1.18 |
| (±sd) | | (11.4) | (11.3) | (0.48) | (0.44) |
| *Relative to (1)* | | *100* | *100* | *100* | *96* |

# Without viscera

[ab]Values with different superscripts in the same row are different (P<0.05)

*Appendix 22. Weight (g) of Slaughtered Bird and their Different Parts After Overnight Cooling (4$^0$C)*

| **Code** | **(1)[a]** | **(3)[a]** | **(1)[a]** | **(3)[a]** |
|---|---|---|---|---|
| *Semduramycin Day 1-35* | ----- | *25* | ----- | *25* |
| *(mg kg$^{-1}$ feed) Day 36-43* | ----- | ---- | ----- | ---- |
| | **Weight in gram** | | **% carcass weight** | |
| **Carcass (including giblets)** | **1933.8** | **2014.5** | **-** | **-** |
| **(±sd)** | **(166)** | **(157)** | | |
| ***Relative to (1)*** | *100* | *104* | | |
| **Breast total** | **512.6** | **546.3** | **26.5** | **27.1** |
| **(±sd)** | **(52.5)** | **(57.8)** | **(1.2)** | **(1.9)** |
| ***Relative to (1)*** | *100* | *107* | *100* | *102* |
| Skin, (±sd) | 51.6 (7.3) | 51.2 (8.2) | 2.7 (0.4) | 2.5 (0.4) |
| *Relative to (1)* | *100* | *99* | *100* | *95* |
| Small muscle, (±sd) | 87.9 (11.8) | 95.2 (12.6) | 4.5 (0.4) | 4.7 (0.4) |
| *Relative to (1)* | *100* | *108* | *100* | *104* |
| Large muscle, (±sd) | 373.1 (42.2) | 399.9 (44.2) | 19.3 (1.0) | 19.8 (1.5) |
| *Relative to (1)* | *100* | *107* | *100* | *103* |
| Breast muscle (total), (±sd) | 460.9[a] (51.1) | 495.1[b] (54.4) | 23.8 (1.1) | 24.6 (1.8) |
| *Relative to (1)* | *100* | *107* | *100* | *103* |
| **Legs and thighs (total)** | **533.5** | **556.9** | **27.6** | **27.7** |
| **(±sd)** | **(45.8)** | **(51.3)** | **(1.8)** | **(1.6)** |
| ***Relative to (1)*** | *100* | *104* | *100* | *104* |
| Skin, (±sd) | 68.7 (8.0) | 71.7 (9.9) | 3.6 (0.4) | 3.6 (0.4) |
| *Relative to (1)* | *100* | *104* | *100* | *100* |
| Fat, (±sd) | 17.0 (6.3) | 18.8 (6.8) | 0.9 (0.3) | 0.9 (0.3) |
| *Relative to (1)* | *100* | *111* | *100* | *100* |
| Bone, (±sd) | 89.6 (12.4) | 90.9 (11.2) | 4.6 (0.6) | 4.5 (0.5) |
| *Relative to (1)* | *100* | *101* | *100* | *97* |
| Muscle, (±sd) | 356.8 (34.6) | 375.0 (39.4) | 18.5 (1.4) | 18.6 (1.3) |
| *Relative to (1)* | *100* | *105* | *100* | *101* |
| **Muscle (breast, legs and thighs),** | **817.8[a]** | **870.1[b]** | **42.3** | **43.2** |
| **(±sd)** | **(78)** | **(84)** | **(1.8)** | **(2.2)** |
| ***Relative to (1)*** | *100* | *106* | *100* | *102* |
| *Wings* | **181.7** | **188.2** | **9.4** | **9.4** |
| **(±sd)** | **(18.5)** | **(12.9)** | **(0.6)** | **(0.5)** |
| ***Relative to (1)*** | *100* | *104* | *100* | *100* |

bw- body weight

[1] Viscera: stomach, intestine, liver, spleen, lung and heart

[ab] Values with different superscript in the same row are different (P<0.05)

*Appendix 23. Sensory Characteristics of Broilers Slaughtered at Days 40 to 43(5-8 days after semduramicin withdrawal; overnight cooling at 4$^0$C)*

| **Code** | | | | **(1)** | | **(3)** |
|---|---|---|---|---|---|---|
| *Semduramycin, Day 1-35* | | | | ----- | | *25* |
| *(mg kg$^{-1}$ feed), Day 36-43* | | | | ----- | | ---- |
| Carcass eviscerated, (±sd) | | | | 1933.8 (166.2) | | 2014.5 (156.9) |
| *Relative to (1)* | | | | *100* | | *104* |
| Dressing percentage, (±sd) | | | | 78.0 (2.3) | | 78.4 (1.9) |
| *Relative to (1)* | | | | *100* | | *100* |
| Juiciness, (±sd) | | | | 5.0 (0.5) | | 5.1 (0.4) |
| *Relative to (1)* | | | | *100* | | *101* |
| Tenderness, (±sd) | | | | 5.1 (0.5) | | 5.2 (0.5) |
| *Relative to (1)* | | | | *100* | | *102* |
| Aroma/flavour, (±sd) | | | | 4.0$^a$ (0.6) | | 4.5$^b$ (0.8) |
| *Relative to (1)* | | | | *100* | | *112* |
| General impression, (±sd) | | | | 4.5 (0.4) | | 4.7 (0.5) |
| *Relative to (1)* | | | | *100* | | *106* |
| Unpleasant pungent flavour, (±sd) | | | | 3.0 (0.0) | | 3.0 (0.0) |
| *Relative to (1)* | | | | *100* | | *100* |
| Scoring scheme | | | | | | |
| **Criteria** | **6** | **5** | **4** | **3** | **2** | **1** |
| Juiciness | Very juicy | Juicy | A little juice | A little dry | Dry | Very dry |
| Tenderness | Very tender | Tender | A little tender | A little tough | Tough | Very tough |
| Aroma/flavour | Excellent | Very good | Good | Satisfactory | Sufficient | Not sufficient |
| General Impression | Excellent | Very good | Good | Satisfactory | Sufficient | Inadequate |
| Unpleasant pungent flavour | ------ | ------ | ------ | None | A little | Strong |

$^{ab}$Values with different superscripts in the same row are different (P<0.05); Absence of superscript indicated lack of significance differences(p>0.05)

*Appendix 24. Sensory Characteristics of Carcass Slaughtered at Days 40 to 43(5-8 days after semduramicin withdrawal; overnight cooling at 4°C)*

| **Code** | | | **(1)** | | | | | **(3)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Semduramicin Day 1-35* | | | ----- | | | | | *25* | | | | |
| *(mg kg$^{-1}$Feed) Day 36-43* | | | ----- | | | | | ---- | | | | |
| Carcass eviscerated | | | 1933.8 | | | | | 2014.5 | | | | |
| (±sd) | | | (166.2) | | | | | (156.9) | | | | |
| *Relative to (1)* | | | *100* | | | | | *104* | | | | |
| Dressing percentage | | | 78.0 | | | | | 78.4 | | | | |
| (±sd) | | | (2.3) | | | | | (1.9) | | | | |
| *Relative to (1)* | | | *100* | | | | | *100* | | | | |
| Scoring | 6 | 5 | 4 | 3 | 2 | 1 | 6 | 5 | 4 | 3 | 2 | 1 |
| Juiciness | 6 | 12 | 2 | 0 | 0 | 0 | 5 | 15 | 0 | 0 | 0 | 0 |
| Tenderness | 4 | 13 | 3 | 0 | 0 | 0 | 7 | 11 | 2 | 0 | 0 | 0 |
| Aroma/flavour | 0 | 5 | 11 | 4 | 0 | 0 | 1 | 11 | 6 | 1 | 1 | 0 |
| General impression | 1 | 8 | 11 | 0 | 0 | 0 | 2 | 14 | 3 | 1 | 0 | 0 |
| Unpleasant pungent flavour | - | - | - | 20 | 0 | 0 | - | - | - | 20 | 0 | 0 |

| **Criteria** | **6** | **5** | **4** | **3** | **2** | **1** |
|---|---|---|---|---|---|---|
| Juiciness | Very juicy | Juicy | A little juice | A little dry | Dry | Very dry |
| Tenderness | Very tender | Tender | A little tender | A little tough | Tough | Very tough |
| Aroma/ Flavour | Excellent | Very good | Good | Satisfactory | Sufficient | Not very sufficient |
| General Impression | Excellent | Very good | Good | Satisfactory | Sufficient | Inadequate |
| Unpleasant pungent flavour | ------ | ------ | ------ | None | A little | Strong |

*Appendix 25. Frequency of Unpleasant Aroma in Broilers Slaughtered at Days 40 to 43; (5-8 d after semduramicin withdrawal; overnight cooling at $4^0$C)*

| **Code** | | | **(1)** | | | **(3)** |
|---|---|---|---|---|---|---|
| Semduramycin , Day 1-35 | | | ----- | | | 25 |
| *(mg $kg^{-1}$ feed),* Day 36-43 | | | ----- | | | ---- |
| Carcass eviscerated | | | 1933.8 | | | 2014.5 |
| (±sd) | | | (166.2) | | | (156.9) |
| *Relative to (1)* | | | *100* | | | 104 |
| Dressing percentage | | | 78.0 | | | 78.4 |
| (±sd) | | | (2.3) | | | (1.9) |
| *Relative to (1)* | | | *100* | | | *100* |
| Criteria | ID* | Panel (n/of n) | Aroma/ Flavour | ID* | Panel (n/of n) | Aroma/ flavour |
| Slightly sour | 1200 | 1/3 | 3.7 | 1206 | 1/3 | 2.3 |
| | 1201 | 1/3 | 4.2 | 1215 | 1/3 | 5.0 |
| | 1210 | 1/3 | 4.1 | 1218 | 1/3 | 3.9 |
| | 1213 | 1/3 | 4.0 | 1264 | 3/3 | 3.9 |
| | 1249 | 4/4 | 4.0 | 1266 | 1/3 | 4.8 |
| | 1251 | 2/4 | 3.4 | 1267 | 1/3 | 4.6 |
| | 1253 | 2/4 | 4.6 | - | - | - |
| Metallic test | 1199 | 1/3 | 3.7 | 1206 | 3/3 | 2.3 |
| | 1212 | 2/3 | 4.3 | 1218 | 3/3 | 3.9 |
| | 1251 | 4/4 | 3.4 | 1256 | 3/4 | 4.1 |
| | - | - | - | 1264 | 3/3 | 3.9 |
| Tasteless | - | - | - | 1268 | 3/3 | 3.6 |

* ID-No. of the broilers

## *Acknowledgements*

First of all, I would like to thank Mr. Prof. Dr. Jürgen M. Gropp for his kind supervision to conduct all experiments and writing the dissertation. Not only that he provided all official support concerning scholarship and registration in his Institute of Animal Nutrition, Nutrition Diseases and Dietetics of the University of Leipzig.

I would also like to thank Mr. Prof. Dr. Dr. Dr. h.c Winfried Drochner, Institute of Animal Nutrition of the University of Hoheinheim, who was extremely responsible to supervise my dissertation for PhD. Mr. prof. Dr. Md. Jasimuddin Khan, Dept. of Animal Nutrition, Bangladesh Agricultural University (BAU) provided all administrative support for me to stay in Germany under study leave from BAU. Mrs. PD. Dr. Annette Schuhmacher was very much responsible to observe my experiment throughout the period and always ready to help me to overcome any difficulties.

I am also grateful to Mr. Prof. Dr. Fritz Rupert Ungemach, because he provided space for me at the Institute of Animal Nutrition, Nutrition Diseases and Dietetics of the University of Leipzig after completion of my research till obtaining PhD. I also like to acknowledge Mr. Prof. Dr. Heinz -Adolf Schoon, because his laboratory (Institute of Pathology, University of Leipzig) was responsible for pathological examinations of selected birds from all the experiments. He also provided me space to continue a part of my research and financial support as an employer.

I would like to acknowledge German Academic Exchange Service (DAAD) for their financial support as scholarship. I also thanks to the authority of Humintech GmbH, Düsseldorf, Germany and Phibro Animal Health, Rixensart, Belgium due to their financial support to conduct my second and third experiment consequently.

Laboratory of the Free State of Saxony, Gustav-Kühn-Straße 8, D-04159 Leipzig, Germany was responsible for the analysis of starch, sugar, amino acids of feeds and composition of meat as well as to determine carcass and sensory characteristics of dressed birds. Semduramicin content in feed and tiamulin content in water was analysed by the Institute Européen de l'Environnement de Bordeaux, Direction Agroalimentaire, 1 Rue du Professeur Vezes, F-33300 Bordeaux, France. Institute of Pathology of the University of Leipzig was responsible for pathological study of birds. Tests for coccidian was conducted at the Institute of Parasitology of the same university. Suspected birds having infectious diseases were examined Landesuntersuchungsanstalt, Wiederitzsch, Germany, for bacteriological and virological test. Blood profiles were conducted at Large Animal Clinic for Internal Medicine of the University of Leipzig. The Clinic for Birds and Reptiles of the university was responsible for the determination of haematocrit and differential blood count. I am highly grateful to the authorities of mentioned institutes due to their cooperation regarding these findings.

My personal thanks go to my wife Mrs. Sufia Afrin Khan (Chumki) and affectionate daughter Fatin Anzum Khan (Tusti) for their care, support and patience during stay in Germany.

(Khan Md. Shaiful Islam)

***Erklärung***

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig angefertigt, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen als solche kenntlich gemacht habe.

Hohenheim, den July 20. 2005 (Khan Md. Shaiful Islam)

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| B Sc in Animal Husbandry | Bangladesh Agricultural University | 1993 |
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2. Award for postgraduate study from German Academic Exchange Service (DAAD)
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4. Talent Scholarship during masters study for very good result in B. Sc in Animal Husbandry

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| Technical Report Writing and Presentation | Graduate Training Institute (GTI), BAU, Mymensingh | 17 – 29May, 1997 |
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2. M. Shahjalal, M.J. Khan and **K.M.S. Islam**. 1996. Effect of *Rhizobium* inoculation and Phosphate application on yield, chemical composition and nutritive value on Sunnhemp *(Crotalaria juncea*) forage in sheep. *Bangladesh J. Training and Devt.* 9(2)75-78.
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11. **K.M.S.Islam**. (2001). Compositional profile of milk round the year at Baghabarighat milk shed area of Bangladesh. *Indian J. of Animal Sciences*. 71(10): 989-991.

12. **K.M.S. Islam** (2002). Feasibility of duckweed as poultry feed (A review). *Indan J. Animal Science.* 72(3)): 67-72

13. **K.M.S. Islam,** A. Schuhmacher, J.M. Gropp and H.-A. Schoon. (2005). Yield, Carcass and Sensory Characteristics of Broiler Meat after Withdrawal of Anticoccidial Semduramicin from Feed. *Pakistan Journal of Nutrition*. 4(3):In press.

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