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Inclusion of oregano oil and a probiotic feed additive into drinking water of broilers – effects on performance and gut health

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I dedicate my work to my family

SUMMARY

Inclusion of oregano oil and a probiotic feed additive into drinking water of broilers – effects on performance and gut health

The hypothesis of this study was that the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would (1) not negatively affect feed and water intake, (2) improve growth performance and (3) positively impact on gut health characteristics, of broiler chickens during a 42-day growth period. Ross 308 chicks (mixed-sex; mean body weight 45.1 g (standard deviation 1.04 g) were randomly assigned to four experimental groups at the start of the experiment: control (without supplement), probiotic (continuous supply of an *E. faecium* commodity [minimum activity per kg: 3.3×10^{12} colony forming units] with drinking water at 200 mg/L), oregano oil (75,000 mg/kg of product; first three days of each week at 0.2 mL/L drinking water), and oregano oil-probiotic (addition of oregano oil (0.2 mL/L) for three days and *E. faecium* commodity (200 mg/L) for four days to drinking water), each group with 10 replicates and each replicate with 10 chicks. Water and feed intakes were determined on a pen basis during the 42-day period. Moreover, body weight gain and feed conversion ratio were calculated to determine overall performance, and weights of liver and abdominal fat pads were assessed. Gut health characteristics encompassed excreta pH and dry matter content, and selected histomorphological parameters, i.e., villus height, crypt depth, villus height to crypt depth ratio, and crypt width.

Water intake was not affected by additives supplied in drinking water. Feed intake was also not different between the groups during the trial, only a slightly higher feed intake was determined in the oregano oil-probiotic group in the finisher phase (days 14-42). Congruently, the water to feed intake ratio was not influenced by additives in drinking water. The data showed a strong correlation between water and feed intakes. Different additives supplied with drinking water did not affect performance of broiler chickens or liver and abdominal fat pad weights. Excreta pH and dry matter content were unaffected by dietary treatment. The probiotic and oregano oil-probiotic supplements affected histomorphological parameters; the villus height in the ileum was higher compared with the other experimental groups. Moreover, in the ileum of broilers in the oregano oil-probiotic group, the crypt depth was deeper and the villus height to crypt depth ratio was wider than for the other experimental groups. Overall, the oregano oil-probiotic administration in drinking water positively affected selected histomorphological gut characteristics in growing broiler chickens.

Consequently, the administration with drinking water may be a viable way to supply these types of feed additives to diets of young chicken without compromising their acceptance to consume water or negatively affecting performance and gut health characteristics. Future studies should be conducted applying varying concentrations of the additives supplied with drinking water.

ZUSAMMENFASSUNG

Effekte einer Verabreichung von Oreganoöl und eines probiotischen Futterzusatzstoffes im Tränkwasser von Masthühnern – Auswirkungen auf Leistungsmerkmale und die Darmgesundheit

Die Hypothese der Studie war, dass die Verabreichung von Oreganoöl und einem probiotischen *Enterococcus faecium*-Stamm im Tränkwasser, entweder einzeln oder alternierend, (1) keine Beeinträchtigung der Futter- und Wasseraufnahme wachsender Masthühner während einer 42-tägigen Mastperiode verursacht, (2) über diesen Zeitraum die Wachstumsleistung erhöht und (3) günstige Auswirkungen aufMerkmale der Darmgesundheit hat. Gemischtgeschlechtliche Küken der Herkunft Ross 308 (mittlere Lebendmasse 45.1 g (Standardabweichung 1.04 g) wurden zu Versuchsbeginn zufällig auf vier Versuchsgruppen verteilt: Kontrolle (ohne Futterzusatzstoff im Tränkwasser), Probiotikum (kontinuierliche Gabe eines *E. faecium*-Produkts [Mindestaktivität pro kg: 3.3×10^{12} koloniebildende Einheiten] im Tränkwasser [200 mg/L]), Oreganoöl (75,000 mg/kg Produkt; jeweils an den ersten drei Tagen jeder Woche [0.2 mL/L Tränkwasser]) und Oreganoöl-Probiotikum (Zugabe über das Tränkwasser von Oreganoöl [0.2 mL/L] über drei Tage und das *E. faecium*-Produkt [200 mg/L] über 4 Tage/Woche). Jede Versuchsgruppe wurde in 10-facher Wiederholung geprüft und jede Wiederholung bestand aus 10 Küken. Der Wasser- und Futterverbrauch wurde über 42 Tage quantifiziert und die Körpermassezunahmen sowie der Futteraufwand wurden als Leistungsmerkmale ermittelt. Zusätzlich wurden Leber- und Abdominalfettpolstermassen ermittelt. Als Merkmale der Darmgesundheit wurden der pH-Wert der Exkreta und deren Trockenmassegehlt ermittelt sowie ausgewählte histomorphologische Parameter erfasst, namentlich Höhe der Darmzotten, Kryptentiefe, das Verhältnis dieser beiden Größen und die Kryptenbreite.

Der Wasserverbrauch wurde durch die Zulagen im Tränkwasser nicht verändert. Auch der Futterverbrauch aller Versuchsgruppen war ähnlich, nur die Oreganoöl-Probiotikum-Gruppe wies einen geringfügig höheren Futterverbrauch ab der dritten Versuchswoche (Tage 14-42) auf. Entsprechend war auch das Verhältnis von Wasser- zu Futterverbrauch in allen Versuchsgruppen übereinstimmend und Wasser- und Futterverbrauch wiesen eine enge Korrelation über den gesamten Versuchsverlauf auf. Die Wachstumsleistung der Masthühner sowie Leber- und Abdominalfettpolstermassen wurden nicht durch die Zulagen im Tränkwasser beeinflusst ($P>0.05$). Der pH-Wert der Exreta und ihr Trockenmassegehalt wurden nicht durch die Zulagen im Tränkwasser verändert. Die Zulage des Pobiotikms und der Kombination Oreganoöl-Probiotikum bewirkte eine größere Höhe der Darmzotten im Ileum. Für die Kombination Oreganoöl-Probiotikum wurde auch eine ausgeprägtere Kryptentiefe ermittelt, die sich in einem weiteren Verhältnis von Zottenhöhe zu Kryptentiefe widerspiegelte. Insgesamt deuten diese Befunde auf eine günstige Beeinflussung der Darmgesundheit durch die geprüften Zulagen im Tränkwasser hin.

Somit kann die Verabreichung der geprüften Zusätze in den gewählten Konzentrationen zum Tränkwasser als mögliche Verabreichungsform betrachtet werden, ohne dabei die Bereitschaft der Masthühner zur Wasseraufnahme zu beeinträchtigen. Die Leistungsmerkmale sowie Leber- und Abdominalfettpolstermassen waren ebenso nicht von den Zulagen im Tränkwasser beeinflusst. Für die Darmgesundheit konnte für einzelne Merkmale eine günstige Beeinflussung durch die geprüften Zulagen im Tränkwasser ermittelt werden. In weiteren Studien sollte untersucht werden, wie sich die Verabreichung unterschiedlicher Konzentrationen der geprüften Zusatzstoffe im Tränkwasser auf Broiler auswirkt.

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ABBREVIATIONS

ADFI	average daily feed intake
ADG	average daily gain
AGP	antibiotic growth promoters
BW	body weight
BWG	body weight gain
CFA	crude fat
CF	crude fibre
CON	control
CP	crude protein
DM	dry matter
DON	deoxynivalenol
DWI	daily water intake
EO	essential oil
FCR	feed conversion ratio
GIT	gastrointestinal tract
ME	metabolizable energy
NfE	nitrogen-free extract
NSP	non-starch polysaccharides
PFA	phytogenic feed additives
WG	weight gain

CHAPTER 1

Literature review

A healthy gut is essential in broilers for their growth and the provision of healthy products for human consumption (Yeh *et al*., 2019). Moreover, there is a direct relationship between animal performance and a 'healthy' gut, with no obvious definition that includes all the physiological functions of the intestinal tract, including nutrient digestion and absorption, host metabolism and energy generation, a stable microbiome, mucus layer development, barrier function, and mucosal immune responses (Kogut *et al.*, 2017).

For optimum feed intake and proper nutrient absorption, a healthy gastrointestinal tract (GIT) is necessary (Ducatelle *et al*., 2018). A well-functioning and healthy gut is the basis of the optimum performance of the birds. When the gut function and health are impaired, digestion and absorption of nutrients are affected and thus the health and performance of birds will be compromised (Sugiharto, 2016). The maintenance of gut health is complex and relies on a delicate balance between the diet, the commensal microflora and the mucosa, including the digestive epithelium and the overlying mucus layer (Montagne *et al*., 2003). A strategy to support the gut health is use of in-feed antibiotics which have been extensively used in animal production for decades. In addition, antibiotics are used therapeutically to improve the health and well-being of animals. A large portion was used for prophylactic purposes and to promote growth rate and feed conversion efficiency as antimicrobial growth promoters (Huyghebaert *et al*., 2011).

Over the past few decades, no major new types of antibiotics have been produced and almost all known antibiotics are increasingly losing their activity against pathogenic microorganisms and the levels of multi-drug resistant bacteria have also increased (Agyare *et al*., 2018). It is estimated that globally more than 60 % of all antibiotics that are produced are utilized in animal production for two purposes, namely therapeutic and non-therapeutic (Agyare *et al*., 2018). Poultry flocks are raised under intensive conditions and it was necessary to use large amounts of antimicrobials to prevent and treat disease, also as growth promoters, but as a consequence antimicrobial resistant poultry pathogens may contribute to occurrence of treatment failure resulting in economic losses, but also be a source of resistant bacteria/ genes (including zoonotic bacteria) that may cause risk for health of humans (Nhung *et al*., 2017).

Antibiotic resistance among the bacterial pathogens and concerns over their widely use in food animals has gained global interest in limiting antibiotic use in animal agriculture and its importance to find innovative antimicrobials that provide alternatives to conventional antibiotics (Seal *et al*., 2013).

Probiotics were described as substitute for in-feed antibiotics which may serve as the best substitutional option for antibiotics in poultry industry (Alagawany *et al*., 2018).

Moreover, Guo *et al*. (2003) stated that the feed additives of plant origin, called as phytogenic feed additives (PFA) or phytobiotics or phytoadditives are considered to be a better alternative as non-antibiotic growth promoters, even though there are well established non-antibiotic growth promoters such as organic acids and probiotics. The PFA as natural non-antibiotic growth promoters can be used in forms of herbs, spices, essential oils and oleoresins (Yitbarek, 2015).

1. Gastrointestinal tract of poultry

The gut is described by Sugiharto (2016) as an essential organ system which plays a vital role in the food digestion and host defence.

1.1. Microbial ecology of the gastrointestinal tract of broilers

The GIT of chicken is as a harbour of a diverse and complex microbiota which has an essential role in nutrient digestion and absorption, immune system development and pathogen removal (Shang *et al*., 2018). Due to the short poultry GIT, microbiota that grows with relatively short transit time requires unique adaptations to adhere to the mucosal wall and to proliferate (Yadav & Jha, 2019). Wei et al (2013) reported that the microbiota of the GIT is a complex ecosystem predominantly comprised of bacteria, but also contains viruses, archaea, fungi, and protozoa.

Xiao et al (2017) identified main microbial groups in different gut sections in broiler chickens. *Firmicutes* were main phylum in the duodenum, jejunum, ileum, and colon while *Bacteroidetes* was dominated in the cecum. The main microbial genera in all gut sections were *Lactobacillus*, *Enterococcus*, *Bacteroides*, and *Corynebacterium*, with *Lactobacillus* prevalent in the upper gut and *Bacteroides* dominant in the cecum. Based on another report by Stamilla *et al* (2021), the gut microbiota in broilers, the ileum was primarily colonized by the *Escherichia/Shigella* genus, while the upper tract of the intestine was primarily colonized by *Lactobacillus*. The caecum, which has the highest diversity of bacterial species, was dominated by the genera *Clostridium* and *Bacteroides*.

1.1.1. Function of the microbial community of the gastrointestinal tract of broilers

The digestive system is an essential primary source of microorganisms, and different kinds of interaction occur between broilers and their intestinal microbiota. Functions of the microbes are nutrient exchange, immunological modulation, physiology of the digestive system, and the exclusion of pathogens (Clavijo & Flórez, 2018). Bailey (2010) reported that the diverse microbial population of the GIT plays a significant role in animal health by aiding digestion, producing nutrients, protecting against pathogens and maturation of the host immune system. Kogut *et al*. (2020) claimed that intestinal microbiota interacts with the immune system of the intestine in broilers. Li *et al*. (2019) revealed that intestinal microbiota can affect the intestinal morphology of the digestive system in broilers. It has been reported that competitive exclusion is a process in which gut commensal microbiota has a vital role in preventing colonization by pathogens in the GIT of chickens (Lan *et al*., 2005).

1.1.2. Factors affecting the microbial community of the gastrointestinal tract of broilers

Clavijo & Vives (2018) indicated factors which affect the microbiota in the GIT of broilers including age, location of the microbiota in the GIT, diet, and antibiotics. Shang *et al*. (2018) reported that the diversity of the chicken GI microbiota is largely affected by age. Gut dominant microbiota become more complex as broilers age (Lan *et al*., 2005) and the bacterial density of the small intestine increases with age (Rehman *et al*., 2007).

About the location of microbiota, in ceca with a lower passage rate of digesta is favourable to diverse groups of bacteria to have an effect on nutrient utilization and overall health of poultry (Yadav & Jha, 2019).

According to Fuller (1984) and Gong *et al*. (2002), the gut microbial population of chicken mostly consists of gram-positive bacteria, the majority of which are facultative anaerobes from crop to terminal ileum, although caeca additionally harbour strict anaerobes and the main site of bacterial activity are crop and caeca and to a lesser extent, the small intestine.

It has been noted that nutrients in the diet of chickens are able to modulate the growth and establishment of the microbiota, therefore, diet as a factor has a major impact on the microbiota (Gabriel et al., 2006). Moreover, antibiotics have an influence on the gut microbiota (Greene *et al*., 2022).

1.2. Function of the digestive tract of poultry

Several mechanisms are involved in the gut function including transit time, digestive secretions, and digestive enzyme activities, and the combination of all these effects influences nutrient digestibility in the GIT (Puvaca *et al*., 2013). Feed enters the crop to be stored for a short time and fermentation occurs partly by the resident bacteria; in the proventriculus the feed will be mixed with hydrochloric acid and pepsin, and next enters the gizzard where mechanical destruction of the feed into smaller particles occurs (Bailey, 2019). In the small intestine amino acids, monomeric carbohydrates, and fatty acids are absorbed. Finally, after termination of digestion two types of droppings are excreted, namely, cecal and a fecal, with different shapes.

Table 1.1: Different segments of the gut in poultry - transit times of feed and pH in each segment

Segments	**Transit time (minutes)**	**pH**
Crop	10 - 50	5.5
Proventriculus/gizzard	30-90	2.5-3.5
Duodenum	5-10	5-6
Jejunum	20-30	6.5-7
Ileum	50-70	7.0-7.5
Cecum/colon	20-30	8.0

Source: Ravindran (2013)

1.3. General view of common digestive health disorders in poultry

The gut as the largest surface of the body is exposed constantly to the external environment (Lievin-Le Moal & Servin, 2006). Yegani & Korver (2008) reported that the GIT is constantly exposed to a wide range of harmful substances and acts as a selective barrier between the tissues of the bird and its luminal environment.

The lumen of the GIT normally contains feed and its constituents, resident and transient microbial populations, endogenous nutrients, and secretions from the GIT and its accessory organs such as the liver, gallbladder, and pancreas. The GIT must selectively allow the nutrients to cross the intestinal wall into the body while preventing the harmful components of the diet from crossing the intestinal barrier (Korver, 2006). Intestinal health problems are considered as a major issue in the poultry industry (De Meyer *et al*., 2019). Enteric disorders are defined by Hafez (2011) as one of the most significant groups of diseases due to their impact on poultry, causinghigh economic losses in many parts of the world due to increasing mortality rates, medication costs, and feed conversion ratios (FCR) and decreasing in weight gain. Moreover, Hughes (2005) mentioned that metabolic stresses related to diet, environment and management can have a negative influence on the delicate balance among physical, chemical, immunological, and microbiological components of the chicken gut and may also severely hamper growth and FCR in broilers.

Any damage in the gut caused by pathogens will lead to poor gut health and results in a reduction in nutrient utilisation efficiency (Choct, 2009). Weakness of the intestinal integrity has a consequence of increase in bacterial adherence to the mucosa, bacterial translocation, weakness to opportunistic bacterial infection, and misappropriation of nutrients (Adedokun & Olojede, 2019).

Occurrence of infection in the gut caused by, e.g., coccidiosis or necrotic enteritis results in an unhealthy gut and leads to inefficiency in digesting and transporting nutrients in the gut (Choct, 2009). Physiological damage contributes to inflammation, loss, or damage of absorptive epithelial cells, and shortening of the all-important villi. The dynamic nature of the organ rapidly repairs the villi, but often it consists of immature and poorly absorptive epithelial cells and ends to a reduction in absorption of nutrients leading to poor growth and/or stunting, as well as difficulties in maintaining fluid balance, causes diarrhoea and wet litter (Perry, 2006).

Common clinical signs related to enteric disorders include dehydration, diarrhoea, depression, weakness, reduced appetite, huddling, vocalization, emaciation, and feed refusal (Perry, 2006). Fast growing broilers are often affected by diarrhoea in the first weeks of their lives leading to impaired performance or the loss of animals (Mueller *et al*., 2012). In the following, a description of the most contributing factors in the gut disorders is given.

1.3.1. Non-infectious factors in the digestive disorders in poultry

1.3.1.1. Nutritional factors

Gut health and nutrition are closely dependent on one another and optimizing dietary nutrient utilization cannot be achieved unless the gut is in a healthy state (Ferket & Veldkamp, 1999). Bedford & Schulze (1998) reported that some ingredients in monogastric diets contain significant amounts of anti-nutritional factors which reduce their feed value and use. Erdaw & Beyene (2018) and Ohm & Südekum (2024) reported that trypsin inhibitors are anti-nutritional factors with considerable activity in legume grains including soybean but zero amounts in cereal grains. Trypsin inhibitors in poultry diets influences the pancreas and had a negative impact on growth performance and increased occurrence of sub-clinical necrotic enteritis in broiler chickens (Palliyeguru *et al*., 2011) but it can be reduced by either optimum heating ('toasting') or enzyme supplementations. With increasing of raw full-fat soybeans in the diets, weight of the pancreas, and activity of trypsin increased and also villus height and crypt depth were negatively affected by raw soybean in the diet of broilers (Rada *et al*., 2017).

According to Annison & Choct (1991) non-starch polysaccharides (NSP) of cereal grains have anti-nutritive activity in the diets of broiler, and the high levels of arabinoxylans (pentosans) in rye and ß-glucans in barley contribute to the reduced nutritive value. Dietary plant ß-mannans (corn-soybean meal-based diets as well as diets containing guar meal, copra meal, and palm kernel meal) in poultry diets also had negative impacts ranging from high intestinal viscosity and low nutrient digestibility and also adverse effects on innate immune response and microbial proliferation in the gut (Shastak *et al*., 2015).

Dekich (1998) stated that feed-borne toxins may lead to enteric disorders. The most prevalent feed-borne toxins are mycotoxins and biogenic amines. According to Resanovic *et al.* (2009), all poultry species and categories are susceptible to mycotoxins. Susceptibility varies to some extent with type, age and production category of poultry, their living conditions and nutritional status and partly on the type, quantity and time period of ingestion of mycotoxins. The presence of mycotoxins causes health disorders and reduces production performance. Deoxynivalenol as a common *Fusarium* toxin in poultry feed has been reported to have a negative influence on gastrointestinal function and leads to disability in the regulation of poultry immune systems (Awad *et al.*, 2008; Awad *et al.*, 2013). Aguzey *et al*. (2019) stated that mainly cereal grains in poultry feed are contaminated by *Fusarium* mycotoxins and exert different effects ranging from acute, obvious diseases with high morbidity and death to chronic disease, low resistance to pathogens resulting in lesser animal productivity, and may also have negative influences such as damage to intestinal morphology, absorption of nutrients, barrier function, and the natural immune response. Biogenic amines are bioactive materials which are synthesized from amino acids (Smith et al., 2000). Stuart *et al*. (1986) revealed that biogenic amines caused malabsorption syndrome characterized by low feed conversion, clinical signs and lesions including enlarged proventriculus, flaccid gizzard often with erosion, and excess mucus in the digestive tract in broiler chickens.

1.3.1.2. Management and environmental factors

Good rearing management is the first point for healthy and productive birds, and profitable poultry production in agreement with animal welfare including all factors affecting the health of birds. These include several factors such as house structure, climatic conditions (ventilation, temperature, and litter condition), stocking density, feed and water supply, hygienic condition as well as the knowledge and qualification of the stockman as they affect one another and can promote or inhibit the health condition of the flock (Hafez, 2011). Adedokun & Olojede (2019) confirmed that stress is a factor which can cause enteric diseases including leaky gut and GITenteritis in poultry. Dharne (2008) reported a number of stress factors for birds such as handling, transportation, overcrowding, and rapid changes in environment which change the gut environment. Burkholder *et al*. (2008) have shown that acute stressors like 24-h feed withdrawal and 24-h exposure to high temperature (30°C) in poultry production systems can cause alterations in the normal intestinal microbiota and epithelial structure, leading to increased attachment of *Salmonella enteritidis.* According to Tsiouri *et al*. (2015) stocking density has a significant effect on the viscosity of intestinal contents, caecal *Clostridium perfringens* and the percentage of Necrotic enteritis positive birds, and the severity of the necrotic lesions. Chronic heat stress has been shown to decrease production performance of broilers, negatively affects intestinal microarchitecture and also increases adrenal hormone concentrations (Sohail *et al*., 2012). It was noted by Wang *et al*. (2016) that litter management regimens (fresh vs. reused litter) have an influence on the chicken gastrointestinal microbiota and may have an impact on the host's nutritional status and intestinal health.

1.4. Alternatives to antibiotic growth promoters as feed additives to improve gut health in poultry

Alternative products and strategies to improve the maintenance of animal gut health are constantly being investigated in order to prevent or reduce the prevalence of pathogens in livestock (Dobrowolski *et al*., 2019). Numerous antibiotics have been added to poultry diets as growth promoters to increase the growth and improve feed efficiency and to reduce mortality in the poultry industry. Based on Denli & Demirel, (2018) all commonly used feed AGP have been banned and not used in the diets of poultry in certain countries due to greater concerns about the potential for antibiotic resistant strains of bacteria and residues of antibiotics in animal tissues. Ban on AGP was effective since 1 January 2006 (European Union Commission, 2006). Ban on AGP in Europe showed that these agents had significant prophylactic activity and their withdrawal is now associated with a weakening in animal health, including increased diarrhoea, weight loss, and mortality due to *clostridial* necrotic enteritis in broilers (Casewell *et al*., 2003). Moreover, the ban on the use of nutritive antibiotics in Europe and increased consumer awareness have created the need for replacements for AGP, as natural and safe feed additives in order to achieve better production results for farm animals (Frankic *et al*., 2009). Alternatives to antibiotics that improve gut health act by different mechanisms including altering gut pH, maintaining protective gut mucins, selection for beneficial intestinal organisms or against pathogens, rise in fermentation acids, nutrient absorption, and humoral immune response (Ferket, 2003). Strategic use of these alternative compounds will improve growth provided that they are used in a way that complements their modes of action (Ferket, 2003). Functionality of the different segments of the digestive tract can be influenced by the diet and feeding systems and functionality of the digestive tract is likely to have a major impact on response to dietary manipulations such as enzyme and pre- or probiotic additives, and therefore needs to be considered in experimental design and explanation of results (Svihus, 2014). Probiotics are single or mixed cultures of live or viable microorganisms which positively influence the host by improving the balance of their intestinal flora (Fuller, 1989). Advantage of the use of medicinal plants as being safer and cheaper and to use for safe food production as well as a decrease in mortality in animals (Omolere & Alagbe (2020). Essential oils as essential aromatic components of herbs and spices which are used as natural substitutes for AGP in poultry feed because they have antimicrobial, antifungal, antiparasitic, and antiviral properties (Krishan & Narang, 2014). Probiotic, phytobiotic, or combination of the two additives in broiler feed could be as alternatives to antibiotics as growth promoters to improve the performance of broiler production (Alloui *et al*., 2014; Ferdous *et al*., 2019).

1.4.1. Phytoadditives in poultry nutrition

In order to increase the overall performance of poultry, phytogenic feed additives can be used as an alternative to antibiotic feed additives in poultry production (Yitbarek, 2015). Benefits of phytogenic feed additives include (Yitbarek, 2015):

- feed intake improvement,
- digestion stimulation,
- growth performance enhancement,
- decrease in incidence of disease,
- reproductive parameter improvement,
- feed efficiency improvement,
- profitability, and
- litter ammonia emission reduction in poultry house.

As a report by Puvaca *et al.* (2013), over the past fifteen years, phytoadditives in animal nutrition have drawn attention for their potential role as alternatives to AGP. Difficulty arises when comparing different studies using phytoadditive due to the large variation in composition and sourcing, thus the potential biological effects of phytogenic compounds may vary; however, there is a large amount of research data supports a potential role of phytoadditive as natural, non-antibiotic growth promoters in broiler nutrition (Puvaca *et al.*, 2013).

According to a review, the most common herbs and spices used as PFA in poultry diet are including oregano, thyme, garlic, horseradish, chilli, cayenne, pepper, peppermint, cinnamon, anise, clove, rosemary, and sage and beneficial properties of phytogenic compounds attributes to their bioactive molecules which are carvacrol, thymol, cineole, linalool, anethole, eugenol, allicin, capsaicin, allylisothiocyanate and piperine (Yitbarek, 2015). PFAs have antimicrobial, antifungal, antiviral, antitoxigenic, antiparasitic and insecticidal properties (Yitbarek, 2015).

Already Grashorn (2010) reported that in commercial poultry nutrition, mainly whole seeds or extracts of black cumin (*Nigella sativa*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), sage (*Salvia* *officinalis*), thyme (*Thymus vulgaris*) and chilli (*Capsicum annum*) are used separately or in combination as feed additive.

Results of an experiment indicated that the herbal natural feed additive such as oregano, du-sacch, quiponin, garlic and thyme may be used as substitutes to an antibiotic growth promoter in broiler production (Demir *et al*., 2005). It has been noted that the use of phytoadditivess in animal nutrition has been investigated in the form of extracts, mostly essential oils, in a variety of animal species (Karásková *et al*., 2015).

Evaluation of phytogenics in the form of extracts, cold pressed oils, and EO was carried out in a number of animals, but the findings are variable (Upadhaya & Kim, 2017).

Through use of these products, we can have a reduction in the use of antibiotics as growth promoters. The beneficial result of these products will not be obvious, and it depends on a number of other factors involved in the production process (Bjedov *et al*., 2013). Actually, there is currently a great interest in essential oils as additives for animal nutrition, as they have more biological activity than the raw material from which they were extracted (Yitbarek, 2015).

In another study Frankic *et al* (2009) reported that plant extracts are applied in animal nutrition as stimulants for appetite and digestion and influence on other physiological functions and support to maintain healthiness and welfare of the animals what can positively affect their performance. Grashorn, (2010) reported that addition of extracts or essential oils to drinking water is less than feed due to higher water intake than feed intake in broilers (about 1.8:1).

Essential oils as essential aromatic components of herbs and spices are used as natural substitutes for AGP in poultry feed because of its antimicrobial, antifungal, antiparasitic, and antiviral properties (Krishan & Narang, 2014).

1.4.1.1. Different kinds of phytogenic compounds

Windisch *et al*. (2008) classified phytogenic compounds mainly based on origin and processing into four groups include:

1) herbs (flowering, non-woody, and non-persistent plants);
2) spices (herbs with an intense smell or taste commonly added to human food);
3) EOs (volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation); and
4) oleoresins (extracts derived by non-aqueous solvents).

Oregano as herb belongs to the mint family. It is closely related to marjoram, even though the flavours vary widely. The common oregano variety has the scientific (latin) name of *Organum vulgare* (Harini, 2014). Light green leaves of oregano are used as a culinary seasoning either as dry or fresh and its use has elicited sensory effects like pungent, pleasantly bitter, herbaceous, and aromatic (Singletary, 2010).

Researchers reported that *Origanum vulgare* (Oregano) has been used traditionally to treat a variety of disorders. Almost every part of *O.vulgare* (Oregano) including roots, leaves, stem and flower are used in the systems of medicines for the treatment of several diseases (Singh *et al.*, 2018).

Furthermore, Gutiérrez-Grijalva *et al*. (2017) described a variety of herb species classified as oregano that commonly used in folk medicine to treat inflammation-related diseases, respiratory and digestive disorders, headaches, rheumatism, diabetes and others.

According to Gutiérrez-Grijalva *et al*. (2017), the most important phytochemicals in oregano are divided based on hydrophilic and hydrophobic properties into two categories: essential oils and phenolic compounds. Tongnuanchan & Benjakul (2014) reported leave part of oregano contains essential oils.

Essential oils are a combination of aromatic oily liquids derived from plant materials such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots (Oyen & Dung, 1999). Essential oils can be found as natural (vegetable origin) or synthetic form (Mehdi *et al*., 2018). Essential oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure that is called terpenes (Cowan, 1999). It was demonstrated by Gaare *et al*. (2013) that chemically EO contains monoterpenes, sesquiterpenes, alcohols, ethers, aldehydes, esters, and ketones as the main constituents.

According to Leyva-López *et al*. (2017) oregano essential oils are complex natural mixtures as they composed of the main components such as terpenes, generally mono- and sesquiterpenes. The main terpenes found in the different species of oregano are carvacrol, thymol, γ-terpinene, p-cymene, terpinen-4-ol, linalool, β-myrcene, trans-sabinene hydrate, and β-caryophyllene as it is presented in (Figure 1.1).

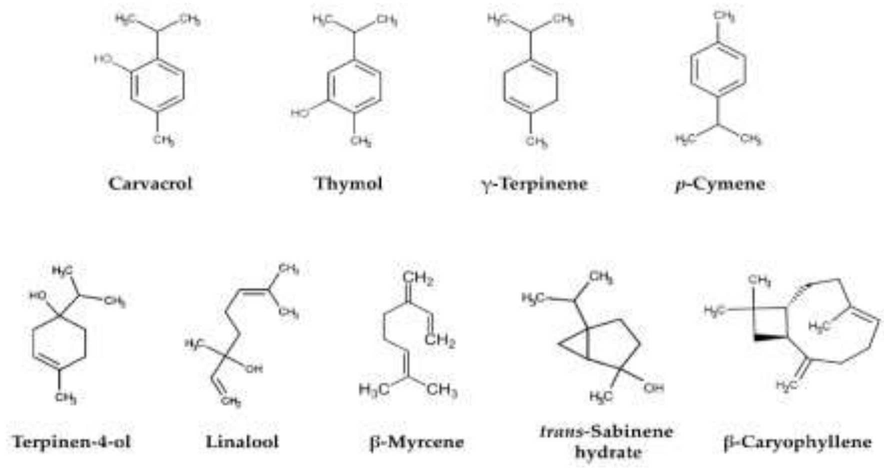

Figure 1.1: Chemical structure of some of the main components of essential oil (EO) in oregano (Leyva-López *et al*., 2017)

Migliorini *et al*. (2019) reported composition of oregano EO obtained by gas chromatography as is presented in (Figure 1.2).

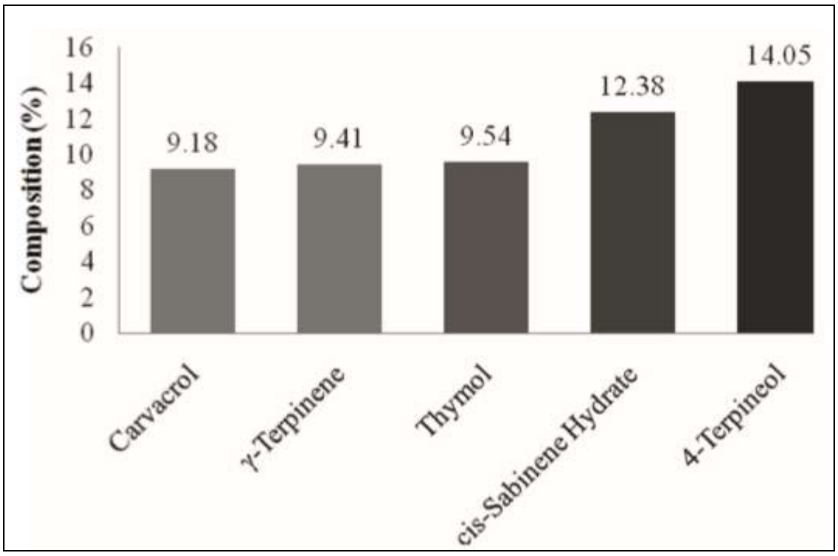

Figure1.2: Major compounds of oregano (*Origanum vulgare*) EO (Migliorini *et al*., 2019)

Among the spices with excellent sources of phenolic compounds, spices such as oregano, thyme, and basil can be mentioned. Phenolic compounds of spices are classified as anthocyanins, flavonoids, and phenolic acids (Kruma *et al*., 2008). Gutiérrez-Grijalva *et al*. (2017) reported that phytochemical compounds in oregano such as flavonoids and phenolic acids contributes partially to health benefits of herbs. Flavonoids and phenolic acids are two of the major and most studied phytochemicals in oregano specie. It was discovered by Vekiari *et al*. (1993) that oregano flavonoids obtained from leaves of oregano as lipid antioxidants including apigenin (flavone), eriodictyol (flavanone) dihydroquercetin (dihydroflavonol), and dihydrokaempferol (dihydroflavonol).

1.4.1.1.1. Biological effects of oregano essential oils

Oregano essential oils are known for their antimicrobial activity, as well as their antiviral and antifungal properties. Also, these compounds have antioxidant, antiinflammatory, antidiabetic activities and cancer inhibitor agents (Leyva-López *et al*., 2017). Some other researchers have also confirmed the properties of oregano EO as anti-microbial (Dorman &. Deans, 2000; Busatta, *et al.*, 2007), anti-fungal (Kocić-Tanackov Sunčica *et al*, 2012), antiviral that carvacrol is an antiviral component in this EO (Vimalanathan & Hudson, 2012; Gilling *et al*, 2014), anti-toxigenic, (Mitchell *et al.*, 2010), anti-oxidant (Stanojević *et al.*, 2016), and anti-inflammatory, as thymol is the isomer of carvacrol has been shown to possess this property activity of oregano EO (Huang & Lee, 2017). Anticoccidial activity of oregano EO in broiler chickens was reported by (Tsinas *et al*., 2011; Alp *et al*. 2012). In addition, Tasdemir *et al*. (2019) reported antiprotozoal activity of oregano EO.

Moreover, it was detected by biological studies that phenolic compounds of *O. vulgare* had antioxidant activity and a weak antiviral activity (Zhang *et al*., 2014). Assiri *et al*. (2016) have shown that oregano's aroma is attributable to the presence of the EO that is accumulated in leaf trichomes. Also, as it has been noted by (Harini, 2014), oregano aroma has a little of bitter taste because of a high concentration of phenolic acids which differs greatly in potency. Sometimes the strains have uniquely mild or very strong tastes. It has been reported that aromatic, slightly bitter taste and functional properties of oregano are related to the quantity and composition of its phenolic components (Karimi *et al*., 2010).

1.4.2. Probiotics

Probiotics are described by World Health Organization (WHO). (2002) as live microorganisms with a benefit for the host healthiness when they are administered in sufficient amounts. According to Dhama *et al.* (2011) probiotics include bacteria, fungi and yeasts and improve the health and well-being of animals or humans. Three basic mechanisms of probiotics include competitive exclusion of pathogenic microbes, production of antibacterial substances (e.g., bacteriocins or colicins) and immune modulation of the host. Fioramonti *et al.* (2003) reported that lactic acid bacteria such as *Lactobacillus, Bifidobacterium and Streptococcus* are the most commonly used probiotics. Specific pH-sensitive bacteria are *E. coli*, *Salmonela* spp., *Listeria monocytogenes*, and *Clostridium perfringens*, whereas *Bifidobacteria* and *Lactobacillus* spp are not pH sensitive (Haque *et al*., 2009). Other researchers reported on species utilized in commercial preparations of probiotic which include *Lactobacillus bulgaricus*, *L. acidophillus*, *L. casei*, *L. helveticus*, *L. salvarius*, *L. plantarum*, *L. faecalis*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Enterobactris faecalis*, *Bifidobacteria species, Saccharomyces cerevisiae, and Toulopsis sphaerica* (Jadhav *et al*., 2015).

It has also been reported that lactic acid bacteria are safe microorganisms able to producing different inhibitory compounds, such as bacteriocins, organic acids as lactic acid, hydrogen peroxide, diacetyl, and carbon dioxide (Vieco-Saiz *et al*., 2019). *Enterococcus faecium* is a lactic acid bacterium that belongs to the physiological intestinal flora of poultry and is used as a probiotic because of its effect on the intestinal microflora through the formation of lactate and short-chain fatty acids (Yu *et al*., 2012). This bacterium competes with pathogenic microorganisms and result in the selective advantage of physiological intestinal bacteria (Kogut *et al*., 2013).

1.4.2.1. Modes of action of probiotics

As described by, e.g., Bermudez-Brito *et al*. (2012), the modes of action of probiotic as feed additives are mainly based on:

- improvement in the epithelial barrier,
- increase in intestinal mucosal adhesion,
- competitive removal of pathogenic microorganisms,
- antimicrobial substance production,
- immune system modulation.

Probiotics increase barrier function through increased mucus production and secretion, antimicrobial peptides, and secretory IgA production, competitive adherence for pathogens, and increase tight junctions' integrity of epithelial cells (Ohland & MacNaughton, 2010) .Adhesion ability to the host as a classical selection criterion of potential probiotic bacteria results in a temporal colonisation which helps to promote immunomodulatory effects and stimulation of gut barrier and metabolic functions (Monteagudo-Mera *et al*., 2019). Probiotic strains can be used as alternative method to effectively decrease the biofilm formation in pathogenic bacteria through competition, elimination and displacement (Woo & Ahn, 2013). It was discovered by a study that bacteriocins produced by *Ent. Faecium* SH 528, *Ent. Faecium* SH 632 and *Ped. Pentosaceus* SH 740 isolated from broiler chickens resulted in an inhibitory activity against enteric pathogens, *L. monocytogenes*, and *Cl. Perfringens* (Shin *et al*., 2008). According to Kang & Im (2015), live probiotics or their metabolites can interact with various immune cells like antigen presenting and T cells to have immunoregulatory functions to maintain immune homeostasis by balancing pro-inflammatory and anti-inflammatory immune responses, although, their effects is different in prevention or modulation of ongoing disease even within a same species.

Alternatives to AGP as probiotics which promote growth or production in the modern meat chicken's industry and benefits of using probiotics include:

- modified host metabolism;
- immuno-stimulation;
- anti-inflammatory reactions;
- exclusion and killing of pathogens in the intestinal tract;
- reduced bacterial contamination on processed broiler carcasses;
- enhanced nutrient absorption and performance; and
- decreased human health risk

(Edens, 2003).

Similarly, Apata (2008) reported that probiotics are increasingly used in animal feed to improve animal productivity and prevention of gastrointestinal infections. Furthermore, in another report probiotics are described as microbial food supplements that have positive effect on the host animal by improving its intestinal microbial balance (Dhama *et al*., 2008), it also improves feed conversion for the target species, reduce morbidity or mortality and have benefits for the consumer by improving quality of product (Musa *et al*., 2009).

In accordance with the previous studies, it has been shown that probiotics can be used as nutritional to promote growth in poultry feeds, modulate intestinal microflora and inhibit pathogen, modulate immune and promote poultry meat quality (Lutful Kabir, 2009). Probiotics as growth promoter in the diet of poultry improved growth performance, feed conversion efficiency, immune responses and was effective against enteric pathogens (Dhama *et al*., 2011). Microbial preparations (probiotics) have also been stated as alternatives to growth promoters of antibiotic type (Thomke & Elwinge, 1998). It has been reported that live apathogenic microbial strains, singly or as multi-strain probiotics belong to the genus *Lactobacillus*, *Streptococcus*, *Bacillus*, *Enterococcus*, *Pediococcus*, *Aspergillus* and *Saccharomyces* used for poultry (Dhama *et al.*, 2011). Research has shown that supplementation of probiotic (Probiolac) at 100 mg/kg diet had an improvement on body weight gain and immune response in broilers up to 4 weeks of age. Additionally, probiotic-fed birds were less vulnerable to the *E. coli* challenge (Panda *et al*., 2000).

1.5. Water intake in poultry

Water is considered as the most important nutrient for poultry. Supply of clean water is essential at all times, and deprivation even for a short period can irreversibly depress growth rates (Ravindran, 2014). Water is involved in many metabolic processes. It has important roles in the digestion and absorption of food, transport of nutrients in the body, and elimination of waste products via urine (Jafari *et al*., 2006). The broiler body consists of 70 % water. It is a main component of the cells, also extracellular environment, and it contributes to the regulation of cellular homeostasis (McCreery, 2015). Leeson *et al*. (1976) indicated that drinking water accounts for 70% of total water intake, with feed and metabolic water accounting for about 10% and 20%. Body water loss is via faeces, urine and evaporative loss from the respiratory tract. Water reabsorption occurs as a result of hypotonicity to extracellular fluid, in the coprodeum and urodeum in the range of 25–30 ml/day. Feed and metabolic water together account for about 20% of total water supply, therefore they should be considered for estimation of the water balance of birds (Leeson & Summers, 2005). Leeson & Summers (2005) reported that the amount of water excreted in the feces and urine is reliant on water intake in broilers and the loss of water is related to considerable variation in the amount and nature of undigested feed. Under normal physiological conditions for adult birds, water intake and output are regulated to ensure a constant level of water in the body. The approximate water consumption in broilers is nearly 1.6 to 2.0 times their feed consumption on a weight basis (Fairchild & Ritz, 2009). Similarly, Collett (2012) reported that birds consume water twice more than feed on a weight basis which underpins the importance of water. Besides, poultry growers can use water consumption to monitor progress of a flock as the simplest and most efficient tool (Watkins & Tabler, 2009). Butcher *et al*. (2009) reported reduction in water consumption as a clinical sign of some diseases in poultry. In addition, Tabler (2003) reported that by knowing water intake, feed intake can be estimated. Based on a report by Aviagen Brief (2018), inadequate water supply contributes to decrease in feed intake and provokes health problems, and changes in water consumption are an early sign of health and performance issues. Besides this, the ratio of water intake to feed intake can be a good way to determine whether or not water consumption is adequate. Water consumption and water to feed intake ratio should be measured daily for a flock to ensure birds are receiving enough water (Aviagen Brief, 2018).

Van der Klis & De Lange (2013) noted that water intake in broilers and laying hens can be highly variable depending on factors such as diet composition, feed form, intestinal health, stress and environmental conditions. Furthermore, Borges *et al*. (2011) noted some additional factors affecting water intake including bird age, environmental temperature, and the level of salts in the diet. Marks & Pesti (1984) reported higher water intake and water to feed intake ratio with increasing crude protein levels in broiler diets. Huang *et al*. (2011) observed that by the addition of increased balanced protein [(the balanced protein levels were 80, 90, 100 and 120%, relative to the Ross 2007 specification (=100%)] in diets, water intake increased. Van Der Klis & De Lange (2013) reported that extra mineral supply in excess to the bird's requirement resulted in higher water intake because more water is needed for eliminating the oversupply through the kidneys as urine output. However, it was demonstrated that water consumption by broilers was not influenced by including up to 600 ppm Fe in drinking water (Fairchild *et al*., 2006).

According to a study by Van der Klis & De Lange (2013), feed form has an influence on water intake in broilers, as dietary factors that increase feed intak such as pelleting also impact on water intak. Van der Klis & De Lange (2013) stated that the effectiveness of water absorption from the intestinal tract is influenced by healthy intestine as weakened intestinal integrity leads to a reduction in net water absorption from the intestinal tract and can lead to diarrhea, whereas excess nutrients will increase water excretion via urine and these two factors consequently stimulate water intake to maintain water balance.

Aviagen Brief (2018) reported that incidence of an intestinal disturbance leads to an increase in water intake during growth in broilers, as it is shown in (Figure 1.3) and therefore any unusual variations in water intake may be an indication of intestinal disorder and as a result recording of water intake is very important. In case of any suddenly increased water intake or prolonged increase in water intake, it is possible to detect, e.g., intestinal disorder in broilers.

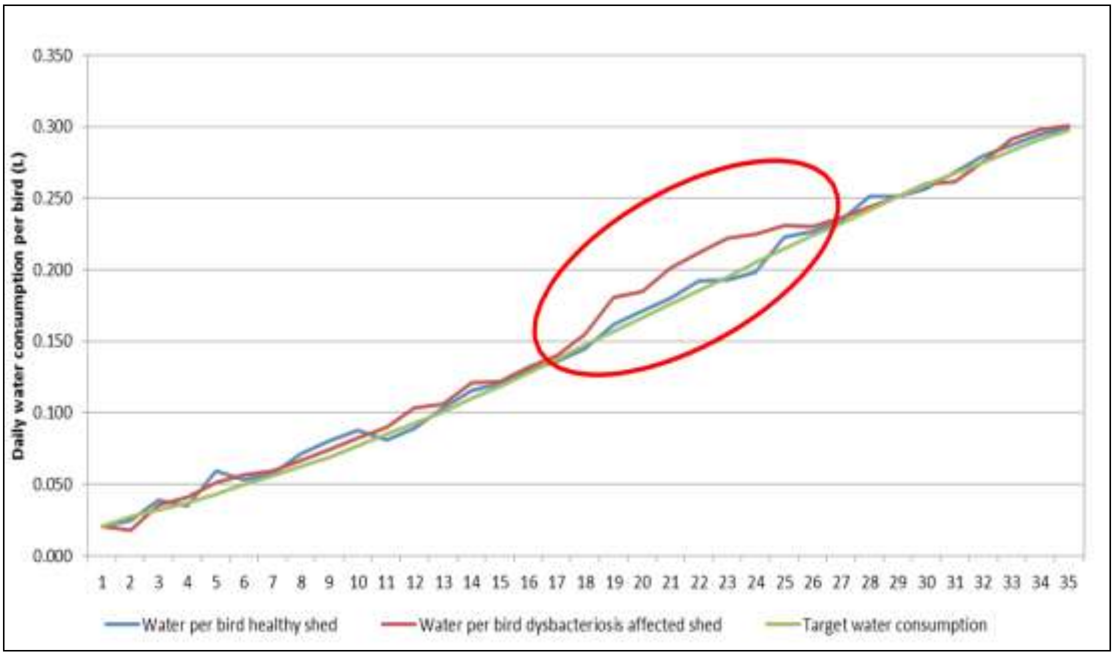

Figure 1.3: Daily water consumption (L) of healthy birds and birds with dysbacteriosis (Aviagen Brief, 2018)

Insufficient water consumption causes a reduction in growth rates. Increased water consumption is associated with higher feed conversions in broilers. Temperature is another factor which affects water consumption in broilers, as the temperature rises, water consumption increases but feed consumption decreases (Vo *et al*., 1978; May & Lott, 1992; Balogun *et al*., 2013). The NRC (1994) reported an increase in water consumption in broilers by approximately 7% for each 1°C above 21°C.

Moreover, the pH of water can have an influence on water consumption in broilers, as the pH range of water to support optimum growth is between 6.0 and 6.8, although broilers tolerate a pH range of 4 to 8. A water pH higher than 8 results in decreased water consumption in broilers (Fairchild & Ritz, 2015). Water consumption in broilers decreases as a percentage of BW (Fairchild & Ritz, 2015). Moreover, because vaccines, vitamins, electrolytes, and antibiotics are offered to the birds through drinking water, it is necessary to estimate the amount of consumed waterof commercially grown broilers. Therefore, having information about the exact amount of water, which is consumed by the birds, is essential to supply the adequate amount of feed additives through drinking water by each bird (Pesti *et al*., 1985).

1.5.1. Effect of phytoadditives on water intake in poultry

Silva-Vázquez *et al*. (2015) reported that supplementation of Mexican oregano oil to broiler diets had a beneficial effect on water intake. Others reported that by the inclusion of some natural feed additives, such as pomegranate peel powder, black pepper powder, and a combination of these two additives in drinking water, an increase was observed of water intake of broiler chickens (Al-Shammari *et al*., 2019). However, on the contrary, the inclusion of *Telfairia occidentalis* (fluted pumpkin) leaf extract at different levels (0, 40, 80, 120, and 160 mL/litre) of drinking water, had no effect on water intake of broilers (Onu, 2012). Adding *Satureja khuzistanica* EO into drinking water, decreased water intake of broiler chickens reared under heat stress conditions. In addition, water to feed intake ratio were reduced (Khosravinia, 2015). Supplementation of natural lavender EO in drinking water did not have any influence on water intake in broiler chickens (Adaszynska-Skwirzynska & Szczerbinska, 2019).

1.5.2. Effect of probiotics on water intake in poultry

It has been demonstrated that by adding probiotic in drinking water, water intake significantly increased in broilers in grower phase. This effect was possibly due to a positive correlation between water intake and feed intake in broilers, because feed intake was increased in broilers in this way (Karimi Torshizi *et al.*, 2010). It was demonstrated that probiotic incorporated into the water had a significant positive effect on water intake in quail (Lokapirnasari *et al.*, 2017).

REFERENCES FOR CHAPTER 1

Adaszyńska-Skwirzyńska, M., & Szczerbińska, D. (2019). The effect of lavender (*Lavandula angustifolia*) essential oil as a drinking water supplement on the production performance, blood biochemical parameters, and ileal microflora in broiler chickens. *Poultry Science*, *98*, 358-365.

Adedokun, S. A., & Olojede, O. C. (2019). Optimizing gastrointestinal integrity in poultry: the role of nutrients and feed additives. *Frontiers in Veterinary Science*, *5*, 348.

Aguzey, H.A., Gao, Z., Haohao, W., Guilan, C., Zhengmin, W., & Junhong, C. (2019). The effects of deoxynivalenol (DON) on the gut microbiota, morphology and immune system of chicken–A review. *Annals of Animal Science*, 19, 305-318.

Agyare, C., Boamah, V. E., Zumbi, C. N., & Osei, F. B. (2018). Antibiotic use in poultry production and its effects on bacterial resistance. *In Antimicrobial resistance-A global threat*. IntechOpen.

Alagawany, M., Abd El-Hack, M. E., Farag, M. R., Sachan, S., Karthik, K., & Dhama, K. (2018). The use of probiotics as eco-friendly alternatives for antibiotics in poultry nutrition. *Environmental Science and Pollution Research*, 25, 10611-10618.

Alloui, M. N., Agabou, A., & Alloui, N. (2014). Application of herbs and phytogenic feed additives in poultry production-A Review. *Global Journal of Animal Scientific Research*, 2, 234-243.

Alp, M., Midilli, M., Kocabağlı, N., Yılmaz, H., Turan, N., Gargılı, A., & Acar, N. (2012). The effects of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin G level, and oocyst count in broilers. *Journal of Applied Poultry Research*, 21, 630-636.

Al-Shammari, K. I. A., Batkowska, J., & Zamil, S. J. (2019). Role of pomegranate peels and black pepper powder and their mixture in alleviating the oxidative stress in broiler chickens. *International Journal of Poultry Science*, 18, 122-128.

Annison, G., & Choct, M. (1991). Anti-Nutritive Activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. *World's Poultry Science Journal,* 47, 232 - 242.

Apata, D. F. (2008). Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of Lactobacillus bulgaricus. *Journal of the Science of Food and Agriculture*, 88, 1253-1258.

Assiri, A.M., Elbanna, K., Al-Thubiani, A., & Ramadan, M. F. (2016). Cold-pressed oregano (Origanum vulgare) oil: a rich source of bioactive lipids with novel antioxidant and antimicrobial properties. *European Food Research and Technology,* 242, 1013-1023.

Aviagen Brief. (2018). Water utilization in broilers. p. 7. Accessed September 2018. https://pt.aviagen.com/assets/Tech_Center/Broiler_Breeder_Tech_Articles/English/AviagenBrief-WaterUtilizationInBroilers2018-EN.pdf?global=1

Awad, W. A., Ghareeb, K., Böhm, J., Razzazi, E., Hellweg, P., & Zentek, J. (2008). The impact of the Fusarium toxin deoxynivalenol (DON) on poultry. *International Journal of Poultry Science*, 7, 827-842.

Awad, W., Ghareeb, K., Böhm, J., & Zentek, J. (2013). The toxicological impacts of the Fusarium mycotoxin, deoxynivalenol, in poultry flocks with special reference to immunotoxicity. *Toxins*, 5, 912–925.

Bailey, R.A. (2019). Gut Health in Poultry - The World Within: *Update*. https://aviagen.com › RossNote-GutHealth-2019-EN

Bailey, R.A. (2010). *Intestinal microbiota and the pathogenesis of dysbacteriosis in broiler chickens* (Doctoral dissertation, University of East Anglia). https://core.ac.uk/download/pdf/2778547.pdf

Balogun, A. A. B., Akinseye, F. M., & Agbede, J. O. (2013). Water and feed consumption in broiler birds during a typical hot weather condition in Akure, Ondo State, Nigeria. *International Journal of Biological and Chemical Sciences*, 7, 1119-1125.

Bedford, M. A., & Schulze, H. (1998). Exogenous enzymes for pigs and poultry. *Nutrition Research Reviews*, 11, 91-114.

Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., & Gil, A. (2012). Probiotic mechanisms of action. *Annals of Nutrition and Metabolism*, 61, 160-174.

Bjedov, S., Peric, L., Đukic Stojcic, M., Milosevic, N., & Vasiljevic, M. (2013). Effect of phytogenic additive on broiler chicken performance. *19th European Symposium on Poultry Nutrition*. https://api.semanticscholar.org/CorpusID:36831163.

Borges, S. A., de Oliveira, J. P., da Silva, A. F., & Dos Santos, T. T. (2011). Use of electrolytes for birds-the practice of theory. In *Proceedings of the 22nd Annual Australian Poultry Science Symposium, Sydney, New South Wales, 14-16th February 2011* (pp. 170-183).

Burkholder, K. M., Thompson, K. L., Einstein, M. E., Applegate, T. J., & Patterson, J. A. (2008). Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to Salmonella enteritidis colonization in broilers. *Poultry Science*, 87, 1734-1741.

Busatta, C., Mossi, A. J., Rodrigues, M. R. A., Cansian, R. L., & Oliveira, J. V. D. (2007). Evaluation of Origanum vulgare essential oil as antimicrobial agent in sausage. *Brazilian Journal of Microbiology, 38, 610-616.*

Butcher, G.D., Jacob, J.P., & Mather, F.B. (2009). Common poultry diseases1. https://api.semanticscholar.org/CorpusID:58809169.

Casewell, M., Friis, C., Marco, E., McMullin, P., & Phillips, I. (2003). The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *Journal of Antimicrobial Chemotherapy*, 52, 159-161.

Choct, M. (2009). Managing gut health through nutrition. *British Poultry Science*, 50, 9-15.

Clavijo, V., & Flórez, M. J. V. (2018). The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review. *Poultry Science*, 97, 1006-1021.

Collett, S. R. (2012). Nutrition and wet litter problems in poultry. *Animal feed science and technology*, 173, 65-75.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12, 564-582.

Dekich, M. A. (1998). Broiler industry strategies for control of respiratory and enteric diseases. *Poultry Science*, 77, 1176-1180.

De Meyer, F., Eeckhaut, V., Ducatelle, R., Dhaenens, M., Daled, S., Dedeurwaerder, A., De Gussem, M., Haesebrouck, F., Deforce, D., & Van Immerseel, F. (2019). Host intestinal biomarker identification in a gut leakage model in broilers. *Veterinary Research*, 50, 1-14.

Dharne, H. V. (2008). Maintaining gut integrity. https://www.thepoultrysite.com/articles/maintaining-gut-integrity.

Demir, E., Sarica, E., Ozcan, M. A., & Suicmez, M. (2005). The use of natural feed additives as alternative to an antibiotic growth promoter in broiler diets. *Archiv für Geflugelkunde*, 69, 110-116.

Denli, M., & Demirel, R. (2018). Replacement of antibiotics in poultry diets. *CABI Reviews*, (2018), 1-9.

Dhama, K., Mahendran, M., Tomar, S., & Chauhan, R. S. (2008). Beneficial effects of probiotics and prebiotics in livestock and poultry: the current perspectives. *Intas Polivet*, *9*, 1-12.

Dhama, K., Verma, V., Sawant, P. M., Tiwari, R., Vaid, R. K., & Chauhan, R. S. (2011). Applications of probiotics in poultry: Enhancing immunity and beneficial effects on production performances and health: A review. *Journal of Immunology and Immunopathology*, 13, 1-19.

Dobrowolski, P., Tomaszewska, E., Klebaniuk, R., Tomczyk-warunek, A., Szymanczyk, S., Donaldson, J., Świetlicka, I., Mielnik-blaszczak, M., Kuc, D., & Muszynski, S. (2019). Structural changes in the small intestine of female turkeys receiving a probiotic preparation are dose and region dependent. *Animal* 13, 2773-2781.

Dorman, H. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88, 308-316.

Ducatelle, R., Goossens, E., De Meyer, F., Eeckhaut, V., Antonissen, G., Haesebrouck, F., & Van Immerseel, F. (2018). Biomarkers for monitoring intestinal health in poultry: present status and future perspectives. *Veterinary Research*, *49*, 1-9.

Edens, F. W. (2003). An alternative for antibiotic use in poultry: probiotics. *Brazilian Journal of Poultry Science*, 5, 75-97.

Erdaw, M. M., & Beyene, W. T. (2018). Anti-nutrients reduce poultry productivity: influence of trypsin inhibitors on pancreas. *Asian Journal of Poultry Science, 12*, 14-24.

European Union Commission (2006). Ban on antibiotics as growth promoter in animal feed enters into effect. Regulation 1831/2003/EC on additives for use in animal nutrition, replacing directive 70/524/EEC on additives in feed-stuffs, 22 December, Brussels, Belgium.

Fairchild, B. D., Batal, A. B., Ritz, C. W., & Vendrell, P. F. (2006). Effect of drinking water iron concentration on broiler performance. *Journal of Applied Poultry Research*, 15, 511-517.

Fairchild, B.D., & Ritz, C.W. (2009). Poultry drinking water primer. https://hdl.handle.net/10724/12452.

Fairchild, B. D. & Ritz, C.W. (2015). Poultry drinking water primer. https://extension.uga.edu/publications/detail.html?number=B1301&title=Poultry%20Drinking%20Water%20Primer.

Ferdous, M.F., Arefin, M.S., Rahman, M.M., Ripon, M.M.R., Rashid, M.H., Sultana, M.R., Hossain, M.T., Ahammad, M.U., & Rafiq K. (2019). Beneficial effects of probiotic and phytobiotic as growth promoter alternative to antibiotic for safe broiler production. *Journal of Advanced Veterinary and Animal Research,* 6, 409.

Ferket, P. R. (2003, October). Controlling gut health without the use of antibiotics. In *Proceedings of the 30th Annual carolina poultry nutrition conference* (pp. 57-68). Raleigh, NC: Academic Publishers.

Ferket, P. R., & Veldkamp, T. (1999). Nutrition and gut health of turkeys and broilers. In *Proceedings of the 26th Annual Carolina Poultry Nutrition Conference and Soybean Meal Symposium. Carolina Feed Industry Association, Raleigh, NC* (pp. 5-18).

Fioramonti, J., Theodorou, V., & Bueno, L. (2003). Probiotics: what are they? What are their effects on gut physiology? *Best Practice & Research Clinical Gastroenterology*, 17, 711-724.

Frankic, T., Voljc, M., Salobir, J., & Rezar, V. (2009). Use of herbs and spices and their extracts in animal nutrition. *Acta Argiculturae Slovenica*, 94, 95–102.

Fuller, R. (1984). Microbial activity in the alimentary tract of birds. *Proceedings of the Nutrition Society*, 43, 55-61.

Fuller, R. (1989). Probiotics in man and animals. *The Journal of applied bacteriology*, 66, 365-378.

Gaare, M., Hussain, S. A., Mishra, S.K., & Ram, C. (2013). Natural antimicrobials for preservation of food. *Dairy and Food Processing Industry Recent Trends-Part I, 1st ed.; Biotech Books: New Delhi, India,* 204-230.

Gabriel, I., Lessire, M., Mallet, S., & Guillot, J. F. (2006). Microflora of the digestive tract: critical factors and consequences for poultry. *World's poultry science journal*, 62, 499-511.

Gilling, D. H., Kitajima, M., Torrey, J. R., & Bright, K. R. (2014). Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. *Journal of Applied Microbiology*, 116, 1149-1163.

Gong, J., Forster, R. J., Yu, H., Chambers, J. R., Wheatcroft, R., Sabour, P. M., & Chen, S. (2002). Molecular analysis of bacterial populations in the ileum of broiler chickens and comparison with bacteria in the cecum. *FEMS Microbiology Ecology*, 41, 171-179.

Grashorn, M. A. (2010). Use of phytobiotics in broiler nutrition–an alternative to infeed antibiotics. *Journal of Animal and Feed Sciences*, 19, 338-347.

Greene, G., Koolman, L., Whyte, P., Burgess, C., Lynch, H., Coffey, A., Lucey, B., O'Connor, L., & Bolton, D. (2022). Effect of doxycycline use in the early broiler production cycle on the microbiome. *Frontiers in Microbiology*, 13, 885862.

Guo, F. C., Savelkoul, H. F. J., Kwakkel, R. P., Williams, B. A., & Verstegen, M. W. A. (2003). Immunoactive, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. *World's Poultry Science Journal*, 59, 427-440.

Gutiérrez-Grijalva, E. P., Picos-Salas, M. A., Leyva-López, N., Criollo-Mendoza, M. S., Vazquez-Olivo, G., & Heredia, J. B. (2017). Flavonoids and phenolic acids from Oregano: Occurrence, biological activity and health benefits. *Plants*, 7, 2.

Hafez, H. M. (2011). Enteric diseases of poultry with special attention to Clostridium perfringens. *Pakistan Veterinary Journal*, 31, 175-184.

Haque, M. N., Chowdhury, R., Islam, K. M. S., & Akbar, M. A. (2009). Propionic acid is an alternative to antibiotics in poultry diet. *Bangladesh Journal of Animal Science*, *38*, 115-122.

Harini, N. S. (2014). Oregano: A wonder herb. *Journal of Pharmaceutical Sciences and Research*, 6, 127.

Huang, C. M., & Lee, T. T. (2017). Immunomodulatory effects of phytogenics in chickens and pigs-A review. *Asian-Australasian Journal of Animal Sciences*, *31*, 617.

Huang, K. H., Kemp, C., & Fisher, C. (2011, November). Effects of nutrition on water intake and litter moisture in broiler chickens. In *22 nd Annual Australian Poultry Science Symposium* (Vol. 26).

Hughes, R. J. (2005). An integrated approach to understanding gut function and gut health of chickens. *Asia Pacific Journal of Clinical Nutrition*, *14*.

Huyghebaert, G., Ducatelle, R., & Van Immerseel, F. (2011). An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal*, 187, 182-188.

Jadhav, K., Sharma, K. S., Katoch, S., Sharma, V. K., & Mane, B. G. (2015). Probiotics in broiler poultry feeds: A Review. *Journal of Animal Nutrition and Physiology*, 1, 4-16.

Kang, H. J., & Im, S. H. (2015). Probiotics as an immune modulator. *Journal of Nutritional Science and Vitaminology, 61*(Supplement), S103-S105.

Karásková, K., Suchý, P., & Straková, E. (2015). Current use of phytogenic feed additives in animal nutrition: a review. *Czech Journal of Animal Science*, 60, 521-530.

Karimi, A., Yan, F., Coto, C., Park, J.H., Min, Y., Lu, C., Gidden, J.A., Lay Jr, J.O., & Waldroup, P.W. (2010). Effects of level and source of oregano leaf in starter diets for broiler chicks. *Journal of Applied Poultry Research*, 19, 137-145.

Karimi Torshizi, M. A., Moghaddam, A. R., Rahimi, S. H., & Mojgani, N. (2010). Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. *British Poultry Science*, 51, 178-184.

Khosravinia, H. (2015). Effects of Satureja khuzistanica essential oils in drinking water on mortality, production performance, water intake, and organ weights in broiler chickens reared under heat stress condition. *International Journal of Biometeorology*, 59, 1711-1719.

Kocić-Tanackov, S. D., Dimić, G. R., Tanackov, I. J., Pejin, D. J., Mojović, L. V., & Pejin, J. D. (2012). Antifungal activity of oregano (Origanum vulgare L.) extract on the growth of Fusarium and Penicillium species isolated from food. *Hemijska industrija*, 66, 33-41.

Kogut, M. H. (2013). The gut microbiota and host innate immunity: regulators of host metabolism and metabolic diseases in poultry?. *Journal of Applied Poultry Research*, 22, 637-646.

Kogut, M. H., Lee, A., & Santin, E. (2020). Microbiome and pathogen interaction with the immune system. *Poultry science*, 99, 1906-1913.

Kogut, M. H., Yin XiaoNan, Y. X., Yuan JianMin, Y. J., & Broom, L. J. C. R. (2017). Gut health in poultry. *CABI Reviews*, (2017), 1-7.

Korver, D. R. (2006). Overview of the immune dynamics of the digestive system. *Journal of Applied Poultry Research*, 15, 123-135.

Krishan, G., & Narang, A. (2014). Use of essential oils in poultry nutrition: A new approach. *Journal of Advanced Veterinary and Animal Research*, 1, 156-162.

Kruma, Z., Andjelkovic, M., Verhe, R., Kreicbergs, V., Karklina, D., & Venskutonis, P. (2008). Phenolic compounds in basil, oregano and thyme. *Foodbalt*, 5, 99-103.

Lan, Y., Verstegen, M. W. A., Tamminga, S., & Williams, B. A. (2005). The role of the commensal gut microbial community in broiler chickens. *World's Poultry Science Journal*, 61, 95-104.

Leeson, S., & Summers, J. D. (2005). Commercial poultry nutrition third edition. *Nottingham University Press, UK*, 398.

Leeson, S., Summers, J. D., & Moran, E. T. (1976). Avian water metabolism—A Review. *World's Poultry Science Journal*, 32(2), 185–195. https://doi.org/10.1079/WPS19760003

Leyva-López, N., Gutiérrez-Grijalva, E. P., Vazquez-Olivo, G., & Heredia, J. B. (2017). Essential oils of oregano: Biological activity beyond their antimicrobial properties. *Molecules*, 22, 989.

Li, C.L., Wang, J., Zhang, H.J., Wu, S.G., Hui, Q.R., Yang, C.B., Fang, R.J., & Qi, G.H. (2019). Intestinal morphologic and microbiota responses to dietary Bacillus spp. in a broiler chicken model. *Frontiers in Physiology*, 9, 1968.

Liévin-Le Moal, V., & Servin, A. L. (2006). The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. *Clinical Microbiology Reviews*, 19, 315-337.

Lokapirnasari, W. P., Dewi, A. R., Fathinah, A., Hidanah, S., Harijani, N., Soeharsono, B. Karimah., & Andriani, A. D. (2017). Effect of probiotic supplementation on organic feed to alternative antibiotic growth promoter on production performance and economics analysis of quail. *Veterinary World*, 10, 1508-1514.

Jafari, R. A., Fazlara, A., & Govahi, M. (2006). An investigation into Salmonella and fecal coliform contamination of drinking water in broiler farms in Iran. *International Journal of Poultry Science*, 5, 491-493.

Lutful Kabir, S. M. (2009). The role of probiotics in the poultry industry. *International Journal of Molecular Sciences*, 10, 3531-3546.

Marks, H. L., & Pesti, G. M. (1984). The roles of protein level and diet form in water consumption and abdominal fat pad deposition of broilers. *Poultry Science*, 63, 1617-1625.

May, J. D., & Lott, B. D. (1992). Feed and water consumption patterns of broilers at high environmental temperatures. *Poultry Science*, 71, 331-336.

McCreey, D. H. (2015). Water consumption behaviour in broilers. Graduate Theses and Dissertations. http://scholarworks.uark.edu/etd/1301"uark

Mehdi, Y., Letourneau-montminy, M.P., Gaucher, M.L., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S.K., Cote, C., Ramirez, A.A., & Godbout, S. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition*, 4, 170-178.

Migliorini M.J., Boiago M.M., Roza L.f., Barreta, M., Arno, A., Robazza, W.S., Galvão A.C., Galli, G.M., Machado, G., Baldissera, M.D., Wagner, R., Stefani, Lcm., & Silva A.S. (2019). Oregano essential oil (Origanum vulgare) to feed laying hens and its effects on animal health. *Anais da Academia Brasileira de Ciências*, 91, e20170901.

Mitchell, T. C., Stamford, T. L. M., Souza, E. L. D., Lima, E. D. O., & Carmo, E. S. (2010). Origanum vulgare L. essential oil as inhibitor of potentially toxigenic Aspergilli. *Food Science and Technology*, 30, 755-760.

Montagne, L., Pluske, J. R., & Hampson, D. J. (2003). A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology*, *108*, 95-117.

Monteagudo-Mera, A., Rastall, R. A., Gibson, G. R., Charalampopoulos, D., & Chatzifragkou, A. (2019). Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. *Applied Microbiology and Biotechnology*, *103*, 6463-6472.

Mueller, K., Blum, N. M., Kluge, H., Bauerfeind, R., Froehlich, J., Mader, A., Wendler, K., & Maller, A. (2012). Effects of broccoli extract and various essential oils on intestinal and faecal microflora and on xenobiotic enzymes and the antioxidant system of piglets. *Journal of Animal Sciences*, 2, 78-98.

Musa, H.H., Wu, S.L., Zhu, C.H., Seri, H.I., & Zhu, G.Q. (2009). The potential benefits of probiotics in animal production and health. *Journal of Animal and Veterinary Advances* 8, 313-321.

National Research Council, & Subcommittee on Poultry Nutrition. (1994). *Nutrient requirements of poultry: 1994*. National Academies Press.

Nhung, N. T., Chansiripornchai, N., & Carrique-Mas, J. J. (2017). Antimicrobial resistance in bacterial poultry pathogens: a review. *Frontiers in Veterinary Science*, 4, 126.

Palliyeguru, MW., Rose, SP., & Mackenyie, AM. (2011). Effect of trypsin inhibitor activity in soya bean on growth performance, protein digestibility and incidence of sub-clinical necrotic enteritis in broiler chicken flocks. *British Poultry Science*, 52, 359-367.

Panda, K., Rao, S. R., & Raju, M. V. L. N. (2006). Natural growth promoters have potential in poultry feeding systems. *Journal of Animal Feed Science and Technology*, 10, 23–25.

Perry, G. C. (Ed.). (2006). *Avian gut function in health and disease*. Cabi.

Puvača, N., Stanaćev, V., Glamočić, D., Lević, J., Perić, L., & Milić, D. (2013). Beneficial effects of phytoadditives in broiler nutrition. *World's Poultry Science Journal*, 69, 27-34.

Rada, V., Lichovnikova, M., & Safarik, I. (2017). The effect of soybean meal replacement with raw full-fat soybean in diets for broiler chickens. *Journal of Applied Animal Research*, 45, 112-117.

Ravindran, V. (2013). Feed enzymes: The science, practice, and metabolic realities. *Journal of Applied Poultry Research*, 22, 628-636.

Rehman, H. U., Vahjen, W., Awad, W. A., & Zentek, J. (2007). Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. *Archives of Animal Nutrition*, 61, 319-335.

Resanović, R., Nešić, K., Nešić, V., Palić, T. D., & Jaćević, V. (2009). Mycotoxins in poultry production. *Zbornik Matice srpske za prirodne nauke*, 7-14.

Ohland, C. L., & MacNaughton, W. K. (2010). Probiotic bacteria and intestinal epithelial barrier function. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 298, G807-G819.

Ohm, D., & Südekum, K. H. (2024). Antinutritive Inhaltsstoffe in Körnerleguminosen und deren Verringerung mittels technischer Verfahren-eine Übersicht. *Züchtungskunde*, 96.

Omolere, A. B. M., & Alagbe, J. O. (2020). Probiotics and medicinal plants in poultry nutrition: A Review. *International Journal of Family Medicine and Primary Care*, 1, 1020.

Onu, P.N. (2012). Effect of aqueous extract of Telfairia occidentalis Leaf on the performance and haematological indices of starter broilers. *ISRN Veterinary Science* in 4 pages.

Oyen, L. P. A., & Dung, N. X. (1999). Plant resources of South-east Asia no. 19: Essential-oil plants.

Panda, A. K., Reddy, M. R., Rao, S. R., Raju, M. V. L. N., & Praharaj, N. K. (2000). Growth, carcass characteristics, immunocompetence and response to Escherichia coli of broilers fed diets with various levels of probiotic. *European Poultry Science*, 64, 152-156.

Pesti, G. M., Amato, S. V., & Minear, L. R. (1985). Water consumption of broiler chickens under commercial conditions. *Poultry Science*, 64, 803-808.

Puvača, N., Stanaćev, V., Glamočić, D., Lević, J., Perić, L., & Milić, D. (2013). Beneficial effects of phytoadditives in broiler nutrition. *World's Poultry Science Journal*, 69, 27-34.

Ravindran, V. (2014). Nutrition of meat animals. Poultry. *Encyclopedia of Meat Science*, 2, 463-470.

Seal, B. S., Lillehoj, H. S., Donovan, D. M., & Gay, C. G. (2013). Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. *Animal Health Research Reviews*, 14, 78-87.

Shang, Y., Kumar, S., Oakley, B., & Kim, W. K. (2018). Chicken gut microbiota: importance and detection technology. *Frontiers in Veterinary Science*, 5, 254.

Shastak, Y., Ader, P., Feuerstein, D., Ruehle, R., & Matuschek, M. (2015). ß-Mannan and mannanase in poultry nutrition. *World's Poultry Science Journal*, 71, 161-174.

Shin, M. S., Han, S. K., Ji, A. R., Kim, K. S., & Lee, W. K. (2008). Isolation and characterization of bacteriocin-producing bacteria from the gastrointestinal tract of broiler chickens for probiotic use. *Journal of Applied Microbiology*, 105, 2203-2212.

Silva-Vázquez, R., Meléndez, L., Santellano-Estrada, E., Rodriguez-Muela, C., Villalobos, G., Mendez-Zamora, G., & Hume, M. (2015). Performance of broiler chickens supplemented with Mexican oregano oil (Lippia berlandieri Schauer). *Revista Brasileira de Zootecnia*, 44, 283–289.

Singh, P.K, Kothiyal, P., & Ratan, P. (2018). Pharmacological and phytochemical studies of *Origanum vulgare*: A Review, *International Research Journal of Pharmacy*, 9.

Singletary, K. (2010). Oregano: overview of the literature on health benefits. *Nutrition Today*, 45, 129-138.

Smith, T. K., Tapia-Salazar, M., Cruz-Suarez, L. E., & Ricque-Marie, D. (2000). Feed-borne biogenic amines: Natural toxicants or growth promotors?. *Avances en Nutrición Acuicola*.

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Ijaz, A., Sohail, A., Shabbir, M.Z., & Rehman, H. (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poultry science*, 91, 2235-2240.

Stamilla, A., Ruiz-Ruiz, S., Artacho, A., Pons, J., Messina, A., Lucia Randazzo, C., Caggia, C., Lanza, M., & Moya, A. (2021). Analysis of the microbial intestinal tract in broiler chickens during the rearing period. *Biology*, 10, 942.

Stanojević, L. P., Stanojević, J. S., Cvetković, D. J., & Ilić, D. P. (2016). Antioxidant activity of oregano essential oil (*Origanum vulgare L.*). *Biologica Nyssana*, 7, 131-139.

Stuart, B. P., Cole, R. J., Waller, E. R., & Vesonder, R. E. (1986). Proventricular hyperplasia (malabsorption syndrome) in broiler chickens. *Journal of Environmental Pathology, Toxicology and Oncology*, 6, 369-385.

Sugiharto, S. (2016). Role of nutraceuticals in gut health and growth performance of poultry. *Journal of the Saudi Society of Agricultural Sciences*, 15, 99-111.

Svihus, B. (2014). Function of the digestive system. *Journal of Applied Poultry Research*, 23, 306-314.

Tabler, G. T. (2003). Water intake: a good measure of broiler performance. *Avian Advice*, 5, 7-9.

Tasdemir, D., Kaiser, M., Demirci, B., Demirci, F., & Baser, K. H. C. (2019). Antiprotozoal activity of Turkish Origanum onites essential oil and its components. *Molecules*, *24*, 4421.

Thomke, S., & Elwinger, K. (1998). Growth promotants in feeding pigs and poultry. III. Alternatives to antibiotic growth promotants. In *Annales de Zootechnie*, 47, 245-271.

Tongnuanchan, P., & Benjakul, S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of Food Science*, 79, R1231-R1249.

Tsinas, A., Giannenas, I., Voidarou, C., Tzora, A., & Skoufos, J. (2011). Effects of an oregano based dietary supplement on performance of broiler chickens experimentally infected with Eimeria acervulina and Eimeria maxima. *The Journal of Poultry Science*, 48, 194-200.

Tsiouris, V., Georgopoulou, I., Batzios, C., Pappaioannou, N., Ducatelle, R., & Fortomaris, P. (2015). High stocking density as a predisposing factor for necrotic enteritis in broiler chicks. *Avian Pathology*, 44, 59-66.

Upadhaya, S. D., & Kim, I. H. (2017). Efficacy of phytogenic feed additive on performance, production and health status of monogastric animals–a review. *Annals of Animal Science*, 17, 929.

Van Der Klis, J.D., & de Lange, L.O.E.K. (2013, August). Water intake in poultry. In *Proc. 19th European Poultry Nutrition Symposium., Postdam, Germany.* (pp.102-107).

Vekiari, S. A., Oreopoulou, V., Tzia, C., & Thomopoulos, C. D. (1993). Oregano flavonoids as lipid antioxidants. *Journal of the American Oil Chemists' Society*, 70, 483-487.

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., & Drider, D. (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Frontiers in Microbiology*, 10, 57.

Vimalanathan, S., & Hudson, J. (2012). Anti-influenza virus activities of commercial oregano oils and their carriers. *Journal of Applied Pharmaceutical Science*, 2, 214-218.

Vo, K.V., Boone, M. A., & Johnston, W. E. (1978). Effect of three lifetime ambient temperatures on growth, feed and water consumption and various blood components in male and female leghorn chickens. *Poultry Science*, 57, 798–803.

Wang, L., Lilburn, M., & Yu, Z. (2016). Intestinal microbiota of broiler chickens as affected by litter management regimens. *Frontiers in Microbiology*, 7, 593.

Watkins, S., & Tabler, G. T. (2009). Broiler water consumption. *Avian Advice*, 11, 11-12.

Wei, S., Morrison, M., & Yu, Z. (2013). Bacterial census of poultry intestinal microbiome. *Poultry science*, 92, 671-683.

Windisch, W., Schedle, K., Plitzner, C., & Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science*, 86(suppl_14), E140-E148.

Woo, J., & Ahn, J. (2013). Probiotic-mediated competition, exclusion and displacement in biofilm formation by food-borne pathogens. *Letters in Applied Microbiology*, 56, 307-313.

World Health Organization (WHO). (2002). Food and Agriculture Organization of the United Nations (FAO). Guidelines for the Evaluation of Probiotics in Food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. London Ontario, Canada. 2002; 1-11.

Xiao, Y., Xiang, Y., Zhou, W., Chen, J., Li, K., & Yang, H. (2017). Microbial community mapping in intestinal tract of broiler chicken. *Poultry science*, 96, 1387-1393.

Yadav, S., & Jha, R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *Journal of Animal Science and Biotechnolog*y, 10, 1-11.

Yegani, M., & Korver, D. R. (2008). Factors affecting intestinal health in poultry. *Poultry science*, 87, 2052-2063.

Yeh, H. Y., Line, J. E., & Hinton, A. (2019). Community-level physiological profiling for microbial community function in broiler ceca. *Current Microbiology*, 76, 173-177.

Yitbarek, M. B. (2015). Phytogenics as feed additives in poultry production: a review. *International Journal of Extensive Research*, 3, 49-60.

Yu, L. C. H., Wang, J. T., Wei, S. C., & Ni, Y. H. (2012). Host-microbial interactions and regulation of intestinal epithelial barrier function: From physiology to pathology. *World Journal of Gastrointestinal Pathophysiology*, *3*, 27.

Zhang, X. L., Guo, Y. S., Wang, C. H., Li, G. Q., Xu, J. J., Chung, H. Y., Ye, W.C., Li, Y.L., & Wang, G.C. (2014). Phenolic compounds from *Origanum vulgare* and their antioxidant and antiviral activities. *Food chemistry*, 152, 300-306.

CHAPTER 2

Scope of the thesis

Increased use of antimicrobial growth promoters in poultry and livestock diets has resulted in an increased concern and awareness of bacterial resistance in such animal species (Apata, 2009). It is, therefore, an ongoing worldwide challenge to replace antibiotic feed additives (Lillehoj *et al.,* 2018). Several beneficial applications of phytogenic feed additives as alternatives to antimicrobial growth promoters in the diet or drinking water have been demonstrated in numerous experiments by researchers through testing in a variety of animal species and categories, such as broiler chickens, laying hens and rabbits. Moreover, positive influences of these feed additives on zootechnical parameters, gut health and tissue or organ mass in broiler chickens has been observed. In addition, application of probiotic feed additives has already been examined and results showed positive outcomes regarding effectiveness of these additives on performance, liver and fat weights, and gut health parameters in broilers. Chapter 1 was therefore included in this thesis to provide an overview of the above mentioned topics.

Most studies have focused on application of phytogenic and probiotic feed additives in the diet. Much less information is available on the efficacy of the named additives when supplied with drinking water. Therefore, this study was designed to examine the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, on broiler chickens.

The first manuscript (Chapter 3) followed the hypothesis that the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would not negatively affect feed and water intake of broiler chickens during a 42-day growth period.

The second manuscript (Chapter 4), in the same experiment, tested the hypothesis that the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would improve growth performance of broiler chickens during a 42-day growth period.

The third manuscript (Chapter 5), during the same 42-day growth period, explored, whether the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would impact on gut health characteristics of growing broiler chickens such as excreta pH and dry matter content, and selected histomorphological parameters, i.e., villus height, crypt depth, villus height to crypt depth ratio, and crypt width.

In the General Discussion and Conclusion chapter (Chapter 6) the results of this study were evaluated and discussed taking into consideration diverse and partly inconsistent findings in previous studies.

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The effect of inclusion in drinking water, either singly or alternating, of oregano oil and a probiotic *Enterococcus faecium* strain on feed and water intake of growing broiler chickens

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Summary

The hypothesis of this study was that the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would not negatively affect feed and water intake of broiler chickens during a 42-day growth period. Ross 308 chicks (mixed-sex; mean body weight 45.1 g (standard deviation 1.04 g) were randomly assigned to four experimental groups at the start of the experiment: control (without supplement), probiotic (continuous supply of an *E. faecium* commodity [minimum activity per kg: 3.3×10^{12} colony forming units] with drinking water at 200 mg/L), oregano oil (75,000 mg/kg of product; first three days of each week at 0.2 mL/L drinking water), and oregano oil-probiotic (addition of oregano oil (0.2 mL/L) for three days and *E. faecium* commodity (200 mg/L) for four days to drinking water), each group with 10 replicates and each replicate with 10 chicks. Water and feed intakes were determined on a pen basis during the 42-day period. Water intake was not affected by additives supplied in drinking water. Feed intake was also not different between the groups during the trial, only a slightly higher feed intake was determined in the oregano oil-probiotic group in the finisher phase (days 14–42). Congruently, the water to feed intake ratio was not influenced by additives in drinking water. The data showed a strong correlation between water and feed intakes. Only the alternating supply of oregano oil and a probiotic in drinking water had a slight positive effect on water and feed intake but consistent responses compared to the control were not observed in any supplemented group. Consequently, the administration with drinking water may be a viable way to supply these types of feed additives to diets of young chicken without compromising their acceptance to consume water.

Keywords: oregano oil, probiotic, *Enterococcus faecium*, water intake, feed intake, drinking water, broiler chickens

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Zusammenfassung

Effekte einer Verabreichung von Oreganoöl und eines probiotischen *Enterococcus faecium*-Stammes im Tränkwasser – einzeln oder alternierend – auf Wasser- und Futteraufnahme von Masthühnern.

Die Hypothese der Studie war, dass die Verabreichung von Oreganoöl und einem probiotischen *Enterococcus faecium*-Stamm im Tränkwasser, entweder einzeln oder alternierend, keine Beeinträchtigung der Futter- und Wasseraufnahme wachsender Masthühner während einer 42-tägigen Mastperiode verursacht. Gemischtgeschlechtliche Küken der Herkunft Ross 308 (mittlere Lebendmasse 45.1 g (Standardabweichung 1.04 g) wurden zu Versuchsbeginn zufällig auf vier Versuchsgruppen verteilt: Kontrolle (ohne Futterzusatzstoff im Tränkwasser), Probiotikum (kontinuierliche Gabe eines *E. faecium* Produkts [Mindestaktivität pro kg: 3.3×10^{12} koloniebildende Einheiten] im Tränkwasser [200 mg/L]), Oreganoöl (75,000 mg/ kg Produkt; jeweils an den ersten drei Tagen jeder Woche [0.2 mL/L Tränkwasser]) und Oreganoöl-Probiotikum (Zugabe über das Tränkwasser von Oreganoöl [0.2 mL/L] über drei Tage und das *E. faecium*-Produkt [200 mg/L] über 4 Tage/Woche). Jede Versuchsgruppe wurde in 10-facher Wiederholung geprüft und jede Wiederholung bestand aus 10 Küken. Der Wasser- und Futterverbrauch wurde über 42 Tage quantifiziert. Der Wasserverbrauch wurde durch die Zulagen im Tränkwasser nicht verändert. Auch der Futterverbrauch aller Versuchsgruppen war ähnlich, nur die Oreganoöl-Probiotikum-Gruppe wies einen geringfügig höheren Futterverbrauch ab der dritten Versuchswoche (Tage 14–42) auf. Entsprechend war auch das Verhältnis von Wasser- zu Futterverbrauch in allen Versuchsgruppen übereinstimmend und Wasser- und Futterverbrauch wiesen eine enge Korrelation über den gesamten Versuchsverlauf auf. Nur bei der abwechselnden Verabreichung von Oreganoöl und dem Probiotikum im Tränkwasser deutete sich ein geringfügig höherer Wasser- und Futterverzehr an, jedoch gab es bei keiner der supplementierten Gruppen konsistente Effekte im Vergleich zur Kontrollgruppe. Somit kann die Verabreichung der geprüften Zusätze in den gewählten Konzentrationen zum Tränkwasser als mögliche Verabreichungsform betrachtet werden, ohne dabei die Bereitschaft der Masthühner zur Wasseraufnahme zu beeinträchtigen.

Schlüsselwörter: Oreganoöl, Probiotikum, *Enterococcus faecium*, Wasserverbrauch, Futter- verbrauch, Tränkwasser, Masthühner

1 Introduction

Water can be considered as the most frequently forgotten nutritional factor in poultry nutrition. It is important to provide clean water all the time, and even short-term water deprivation will cause growth rates to decline (RAVINDRAN, 2014). Water must always be available to the animals for *ad libitum* consumption. Moreover, water is involved in many aspects of poultry metabolism, including digestion and absorption of nutrients, transport of nutrients in the body, and elimination of waste products via urine (JAFARI et al., 2006). The animal gets water from three different sources, namely drinking water, water in feed and metabolic water (MCCREERY, 2015). Feed and metabolic water together account for about 20% of total water needs, therefore, as broiler ages fat percentage of body increases and protein content decreases so that the body water content as a percentage of body weight decreases (MCCREERY, 2015). Inadequate water consumption leads to a reduction in growth performance, whereas increase in water cause a wastage of water (MCCREERY, 2015) and can result in wet litter, a causative factor of food pad dermatitis in broilers (VAN DER KLIS and DE LANGE, 2013). On a weight basis, the approximate water consumption of broilers is about twofold the feed consumption (COLLETT, 2012), which additionally underpins the importance of water for birds.

With an increase of the protein content in the diet, the water intake and water to feed ratio increased (MARKS and PESTI, 1984). Moreover, if protein and minerals are oversupplied, enhanced water intake is required to allow an increased elimination of nitrogen, in the form of uric acid, and minerals through the kidney as urine (VAN DER KLIS and DE LANGE, 2013). It has been noted that the pH of the offered water can have an influence on water consumption in broilers, who prefer pH values between 6.0 and 6.8 and can tolerate a pH range of 4 to 8 (FAIRCHILD and RITZ, 2015). The same authors reported that, as birds age, total water consumption rises but it decreases as a percentage of body weight (FAIRCHILD and RITZ, 2015). PESTI et al. (1985) estimated that age-dependant water consumption can be predicted. i.e., water consumption (g) = [5.28 × age (days)]. Temperature is another factor which affects water consumption in broilers, as it is observed that as temperature rises, water consumption increases in broilers, but feed consumption decreases (VO et al., 1978).

Furthermore, as the use of antibiotic feed additives has been banned in many regions worldwide, and, along with the goal of a general reduction of the use of antibiotics in farm animals, has accelerated the exploration of alternative feed additives (LEE et al., 2003; AYALEW et al., 2022). *Enterococcus faecium* belongs to the physiological intestinal microbiota of poultry and has been characterised as a probiotic because of its modulating effect on the intestinal microbiota through the formation of lactate and short-chain fatty acids (YU et al., 2012). This bacterium competes with pathogenic microorganisms and may result in a selection advantage of the physiological intestinal bacteria (KOGUT, 2013). Inclusion of probiotics in feed or water has repeatedly resulted in improved broiler performance similar to supplementation of an antibiotic (avilamycin) (MOUNTZOURIS et al., 2007). Addition of a probiotic to drinking water has increased feed and water intake of broilers in the grower phase and over the whole growth period (KARIMI TORSHIZI et al., 2010).

Other studies have indicated that herbs, spices, and their extracts, often termed as 'botanicals' or 'phytogenics', have a wide range of activities, and among other effects, may stimulate feed intake and intestinal endogenous secretions (WENK, 2003). The supplementation of Mexican oregano oil to a diet led to a beneficial effect on feed and water intake in broilers (SILVA-VÁZQUEZ et al., 2015). Probiotics, phytobiotics, or a combination of these two feed additive types could thus improve the performance of broilers (ALLOUI et al., 2014). Furthermore, probiotic addition via drinking water has led to a stronger improvement of growth performance in broilers compared to the more conventional in-feed method (KARIMI TORSHIZI et al., 2010). Therefore, the objective of this study was to evaluate the effects of different additives supplied in drinking water, on water intake, feed intake, and water to feed intake ratio in growing broiler chicken.

The hypothesis was that the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would not negatively affect feed and water intake of growing broiler chicken. Consequently, this way of administration may be a viable alternative to supplying these types of feed additives to diets of young, rapidly growing chicken without compromising their acceptance to consume water.

2 Material and methods

2.1 Experimental design and broiler management

Four hundred one day-old Ross 308 chicks (mixed sex) were purchased from a commercial hatchery. The initial body weight of each chick was determined at the beginning of the experiment (average (with standard deviation) 45.1 g (1.04 g). The birds were randomly assigned to four dietary treatment groups, namely control, probiotic, oregano oil, and oregano oil-probiotic, each with 10 replicates and each replicate with 10 chicks. The birds were reared in a room from day 0 to 42, housed in pens of the same size, 110 cm × 120 cm for 10 birds. The pen floors were covered with wood shaving litter with a thickness of 5 cm at the beginning of the trial. Dry litter was added as necessary to maintain a low moisture level of the litter and lower the risk for foot-pad dermatitis. The pens were equipped with one bucket nipple drinker (10-L capacity) and one hanging plastic feeder. The nipple drinkers were frequently checked to ensure that no leakage occurred and their position was adjusted to the body size of the birds in regular intervals. All chicken had *ad libitum* access to drinking water and feed during the 42-day trial. Moreover, temperature, ventilation, lighting and vaccination (Newcastle disease) programs followed a protocol established at the research station.

2.2 Experimental treatments

A crumbled starter diet was provided to the birds from day 0 to 7, followed by a pelleted grower diet from day 8 to 14, and a pelleted finisher diet from day 15 to 42. The chemical composition of the diets was analysed in accordance with the methods of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2012) and encompassed dry matter, crude fat, crude fibre, crude protein, and crude ash. Moreover, nitrogen-free extract (NfE) was calculated subsequently. In addition, starch, sugar, calcium, sodium, potassium, and phosphorus concentrations were also determined (VDLUFA, 2012).

The N-corrected apparent metabolizable energy (ME) content was calculated from chemical composition according to WPSA (1984). Data of ingredients and chemical composition of the basal diet are presented in Table 1.

Tab. 1. Ingredients and chemical composition of the diets
Bestandteile und chemische Zusammensetzung der Rationen

	Periods of age		
	Starter (days 0 – 7)	Grower (days 8 – 14)	Finisher (days 15 – 42)
	Ingredients (decreasing order of their proportions)		
	Wheat	Wheat	Wheat
	Corn	Corn	Corn
	Soybean meal	Soybean meal	Soybean meal
	Soybean	Soybean	-
	Rapeseed meal	Rapeseed meal	Rapeseed meal
	Soybean oil	Soybean oil	Soybean oil
	Calcium carbonate	Calcium carbonate	Calcium carbonate
	Mono-calcium phosphate	Mono-calcium phosphate	-
	Sodium carbonate	Sodium carbonate	-
	-	Lignocellulose	Lignocellulose
	Chemical composition (g/kg unless stated)		
ME (MJ/kg)	12.1	12.4	13.3
Dry matter	919	910	912
Crude protein	213	205	175
Crude fat	45.0	58.0	82.0
Crude fibre	55.4	43.5	44.0
Ash	57.2	50.2	38.0
Sugar	33.9	31.4	34.1
Starch	408	407	439
Calcium	10.8	7.00	5.30
Sodium	1.10	1.20	1.10
Potassium	9.50	8.90	7.00
Phosphorus	6.44	5.67	4.57

Abbreviations: ME, N-corrected apparent metabolizable energy (calculated according to WPSA, 1984).

2.3 Sources of probiotic and oregano oil additives

The probiotic used in this study was *E. faecium* (DSM 7134, minimum activity per kg: 3.3×10^{12} colony forming units; Lovit Probiotic; Lohmann Animal Nutrition, Cuxhaven Germany) as water-soluble supplement. This supplement was incorporated constantly into drinking water of the probiotic group at 200 mg/L. Oregano oil (75,000 mg/kg; Lovit Progano Liquid; Kaesler Animal Nutrition, Cuxhaven, Germany) at level of 0.2 mL/L was incorporated into drinking water of the oregano oil group for the first three days per week. The combined treatment group, oregano oil-probiotic, received oregano oil in their drinking water for the first three days and the probiotic the other four days per week. Additives were mixed with drinking water based on calculated drinking water usage (mL) per day. The additive-water mixtures were prepared shortly before they were offered to the birds in the nipple drinkers for each pen.

2.4 Water intake and feed intake and water to feed intake ratio measurements

To quantify daily water intake, the water was offered to the birds every day at 0700 h and the remaining water in the nipple drinkers in each pen was weighed on the next morning at 0700 h with a scale to the nearest gram. These values were divided by the number of birds in each pen to yield averages per pen. The average daily water intake was calculated as the difference between the average quantity of water given to the birds each day and the average quantity of remaining water on the next morning. Similarly, weekly water intake was calculated as the sum of average daily water intake for each week.

Feed intake of broilers was measured for starter, grower, and finisher diets. The offered feed for each pen was weighed separately and recorded at the beginning of each week and then residual feed in the feeders in each pen was weighed at the end of each week at day 7, 14, 21, 28, 35 and 42 of the trial; these values were divided by the number of birds in each pen to yield average values per pen. The average quantity of the offered feed minus the average quantity of the residual feed at the end of each week resulted in the total consumption per week which was divided by seven to calculate the average feed intake per bird and day. For the calculation of the water to feed intake ratio, average water intake was divided by average feed intake.

As an indication of the general health status, daily mortality rate was determined in this way that all diseased and dead birds were removed from the pen, weighed, marked with a description and recorded. Records of removed birds were needed for an accurate calculation of water intake and feed intake which were corrected for mortality records.

For calculation the daily water intake, daily feed intake, and water to feed intake ratio for each period, namely starter, grower, and finisher periods, data in week 1 of the trial were considered as starter period, week 2 as grower period and weeks 3, 4, 5, and 6 were considered as finisher period.

2.5 Statistical analyses

The experimental data were statistically analysed as a completely randomized design using SPSS Software (v 19.0; SPSS Inc, Chicago, IL, USA). Values were checked for normality before conducting an analysis of variance (ANOVA). Data of all variables were subjected to a one-way ANOVA and comparison between treatment (i.e., diet) means was conducted using Duncan's multiple range test (DUNCAN, 1955). P-values < 0.05 were considered as indicating significant differences. A trend was observed when P-values were ≥ 0.05 but < 0.10. A scatter plot was created and then a linear regression analysis was performed to identify relationship between water intake and age as well as feed intake and age.

3 Results

The additives given into drinking water of broilers had no overall effect on average daily water intake in any period of the trial (Tab. 2). In terms of water intake during the starter, grower, and finisher periods, there was a slight reduction in water intake in the oregano oil group during the trial (Tab. 2).

Tab. 2.Effects of supplements added to drinking water on average daily water intake (g/bird) of broilers
Auswirkungen von mit dem Tränkwasser verabreichten Zusatzstoffen auf die durchschnitt-liche tägliche Wasseraufnahme (g/Tier) der Masthühner

Growth periods	*Experimental groups*					
	Control	Probiotic	Oregano oil	Oregano oil-Probiotic	Pooled SEM	*P-value*
Starter phase (days 0–7)	53.8 ± 3.12	53.67 ± 2.07	52.56 ± 2.58	53.66 ± 2.67	0.409	0.702
Grower phase (days 8–14)	106 ± 5.17	105 ± 4.53	101 ± 5.91	106 ± 5.93	0.869	0.262
Finisher phase (days 15–42)	244 ± 8.96	241 ± 10.19	233 ± 13.17	242 ± 11.00	1.79	0.147

Note: Data expressed as means ± standard deviation.
Abbreviation: SEM, standard error of means.

In accordance with the results on water intake, values presented in Table 3 show that feed intake of broilers, in general, was also not affected (P > 0.05) by inclusion of different additives in drinking water. Only few small, insignificant reductions of feed consumption of birds in the oregano oil group compared to the other groups were observed. Broilers in the oregano oil group consumed less feed than broilers the probiotic group in the starter period. In addition, in the finisher period chicken in the oregano oil group had slightly lower feed consumption, whereas on the contrary, chicken in the oregano oil-probiotic group consumed slightly more feed than the other groups.

Tab. 3. Effects of supplements added to drinking water on average daily feed intake (g/bird) of broilers
Auswirkungen von mit dem Tränkwasser verabreichten Zusatzstoffen auf die durchschnitt- liche tägliche Futteraufnahme (g/Tier) der Masthühner

Growth periods	*Experimental groups*					
	Control	Probiotic	Oregano oil	Oregano oil-Probiotic	Pooled SEM	*P-value*
Starter phase (days 0–7)	22.0 ± 0.594	22.5 ± 0.636	21.8 ± 1.10	22.0 ± 1.06	0.140	0.322
Grower phase (days 8–14)	45.0 ± 2.44	45.4 ± 1.65	43.6 ± 3.19	43.8 ± 4.03	0.466	0.485
Finisher phase (days 15–42)	130 ± 3.05	129 ± 5.44	128 ± 5.86	131 ± 5.25	0.788	0.493

Note: Data expressed as means ± standard deviation.
Abbreviation: SEM, standard error of means.

The data presented in Table 4 indicate that the birds did not respond (P > 0.05) to the supplemented additives in terms of water to feed intake ratio. This result is in line with the observations reported above that both, water and feed intake, were not or only marginally affected by inclusion of additives in drinking water. The data indicate that water to feed intake ratio during the starter and grower periods in the probiotic group was slightly lower than in the other groups, and the values were slightly higher in the oregano oil-probiotic group. In the finisher period the water to feed intake ratio was slightly lower in the oregano oil group, and, on the contrary, in the probiotic and control groups the water to feed intake ratio was marginally higher than in the other groups. In general, the water to feed intake ratio in the finisher period was lower than in the other periods.

Tab. 4. Effect of inclusion of different supplements to drinking water on water intake to feed intake ratio (mL/g) of broilers
Auswirkungen von mit dem Tränkwasser verabreichten Zusatzstoffen auf das Verhältnis von Wasser- zur Futteraufnahme (mL/g) von Masthühnern

Growth periods	*Experimental groups*					
	Control	Probiotic	Oregano oil	Oregano oil-Probiotic	Pooled SEM	*P-value*
Starter phase (days 0–7)	2.44 ± 0.085	2.38 ± 0.039	2.42 ± 0.084	2.45 ± 0.129	0.014	0.390
Grower phase (days 8–14)	2.35 ± 0.101	2.31 ± 0.091	2.33 ± 0.116	2.42 ± 0.134	0.018	0.155
Finisher phase (days 15–42)	1.86[a] ± 0.046	1.86[a] ± 0.049	1.81[b] ± 0.047	1.83[ab] ± 0.038	0.008	0.054

Note: Data expressed as means ± standard deviation.
[a,b]Different superscripts indicate significant differences between the values ($p < 0.05$).
Abbreviation: SEM, standard error of means.

Because experimental treatments had no effect on water or feed intake, data were averaged across experimental treatment groups to depict the development of water and feed intake during the 42-day growth period. Both water and feed intake increased continuously and linearly, which indicate undisturbed experimental conditions (Fig. 1).

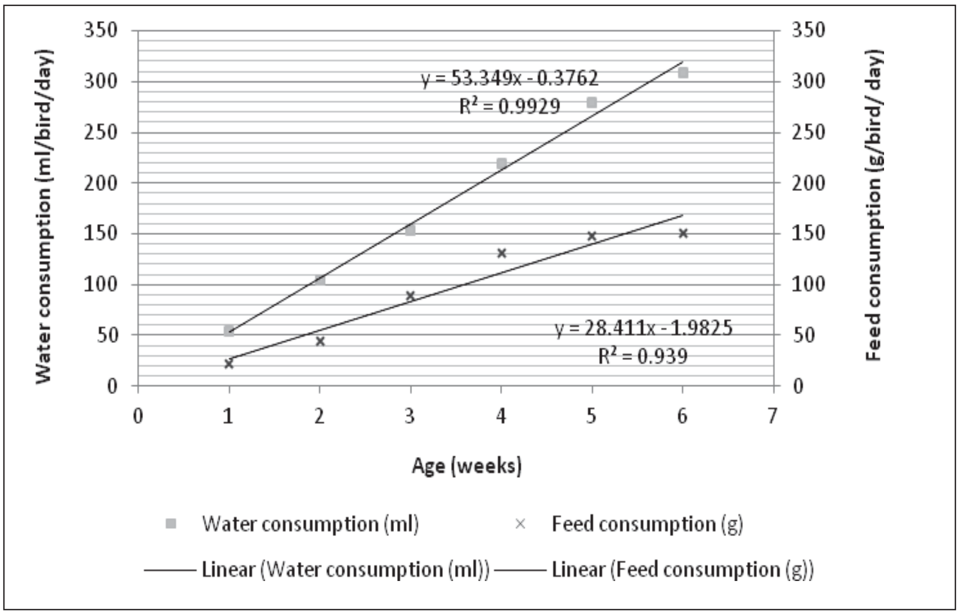

Fig. 1. Development of daily water and feed consumption of broilers during a 6-week (42-days) growth period averaged across four experimental treatments
Entwicklung der täglichen Wasser und Futteraufnahmen von Masthühnern während einer sechswöchigen (42 Tage) Wachstumsperiode im Mittel der vier Versuchsratione

4 Discussion

Alternative products and strategies to maintain or improve animal gut health are constantly being investigated in order to prevent or reduce the prevalence of pathogens in livestock (DO-BROWOLSKI et al., 2019). Numerous antibiotic feed additives have been used in poultry diets over decades to increase growth, improve energy and nutrient use efficiency, and reduce mortality of poultry. Commonly used antibiotic feed additives have been banned and are, therefore, no longer used in poultry diets in many regions worldwide due to significant concerns about the potential for development of antibiotic-resistant bacteria strains and, partly, antibiotic residues in animal tissues (DENLI and DEMIREL, 2018). The ban on the use of nutritive antibiotics in Europe and increased consumer awareness have created the need for replacements for antibiotic feed additives and stimulated the exploration of (near-)natural and safe feed additives in order to maintain or even improve production results for farm animals (FRANKIC et al., 2009). Herbs in various forms (e.g., whole above-ground biomass, leaves, extracts), belonging to the group of phytogenic products (WINDISCH et al., 2008), have long been proposed as alternatives to antibiotics in relation to the productivity of healthy farm animals. Specific herbs have been proposed to regulate feed intake and stimulate digestive secretions which in turn leads to optimized digestion capacity and reduced risk of digestive disorders (WENK, 2003). Phytogenic feed additives have recently gained considerable attention particularly due to their potential to enhance performance mediated through a healthy gut environment (MURUGESAN et al., 2015). In addition, live microbial preparations, i.e., probiotics, have also been stated as alternatives to antibiotic feed additives (THOMKE and ELWINGER, 1998).

In addition, water intake can be evaluated as it is closely related to feed intake and, even- tually, performance in broilers. Based on this relationship, in several studies, different types of feed additives as a replacement for antibiotics have been added to drinking water of broilers and their effects on water intake were studied (KARIMI TORSHIZI et al., 2010; KHOSRAVINIA, 2015; ADASZYŃSKA-SKWIRZYŃSKA and SZCZERBIŃSKA, 2019). KAMPHUES et al. (2019) have systematically evaluated pros and cons of supplementation of feed additives to drinking water and have also noted concerns that distinct additives may change water properties such as nutrient concentrations, sensory attributes and microbial status. These hints should be considered before feed additives are provided with drinking water.

Evaluation of effects of inclusion of oregano oil and a probiotic *E. faecium* strain in drink ing water, either singly or alternating, on feed and water intake of growing broiler chickens is a novel approach to compare different types of feed additives. In this study, only marginal effects were observed, namely water intake of birds in the oregano oil group slightly decreased compared to the control group. Birds have less taste buds than mammals which can be taken as a proof of lower taste acuity (ROURA et al., 2013) which may also explain that the bitterness of oregano oil did not provoke a more pronounced negative effect on water or feed intake. On the contrary to our observations, at supplementation levels of Mexican oregano oil of 200 and 400 mg/kg diet water intake of broilers was improved (CÁZARES-GALLEGOS et al., 2019). In line with our data, LEE et al. (2003) showed that supplementation of feeds with cinnamaldehyde reduced water intake in broilers, yet they claimed that the mechanism causing a decrease in water intake induced by cinnamaldehyde was unidentified.

Water intake in broilers and laying hens can be highly variable depending on factors such as diet composition, feed form, intestinal health, stress, and environmental conditions (VAN DER KLIS and DE LANGE, 2013). Only few studies have been conducted to date to investigate the influence of oregano essential oil on water intake in broilers (Cázares-Gallegos et al., 2019). An effect of extra energy supplied with oregano oil can be largely excluded. To exemplify this statement, based on 200 mg oregano oil per litre of drinking water in week 1 and a daily water intake of 53 mL/bird, only 0.40 kJ ME would have been additionally consumed and it is very unlikely this small amount of extra ME did modify the willingness of birds to drink water or consume their feed.

In this study, feed intake was not remarkably different between treatments. Only feed consumption of birds in the oregano oil group was slightly lower compared with the other groups, which is in line with observations on water intake. Consistent with our result it was also reported that dietary oregano oil (ESCALERA-VALENTE et al., 2016) or oregano essential oil (TEKELI et al., 2006) did not affect feed intake in broilers. Likewise, AGUILAR et al. (2013) showed that by dietary inclusion of essential oil from copaiba (*Copaifera* spp.) feed intake of broiler chicken was not affected. Opposed to these observations, in one study birds fed an oregano essential oil-supplemented diet consumed significantly less feed in comparison with those fed the negative control diet (ALP et al., 2012). On the contrary, feed intake in broilers was positively influenced by Mexican oregano oil at 800 mg/kg inclusion level on day 39 of the trial period (SILVA-VÁZQUEZ et al., 2015). Summarized, these observations underpin that effects of flavourings on chicken performance have not been fully investigated (KHOSRAVINIA, 2015) nor understood. In addition, differences between results of studies might be due to variations in management practices and bird physiological state (KHOSRAVINIA, 2015) and comparisons across studies are often restricted due to inadequate characterization of feed additives and their active compounds.

Consistent with previous studies (CAKIR et al., 2008; OLNOOD et al., 2015; HIDAYAT et al., 2018) and our expectations, we did not observe a consistent effect of administration of a probiotic on feed intake. SHEWITA et al. (2016) reported that *E. faecium* supplementation of broiler diets caused a slight improvement in average daily feed intake which is in accordance with our observations on inclusion of *E. faecium in* drinking water with marginally higher daily feed intake of birds in the probiotic group as compared to the other experimental groups. Others have reported that addition of a probiotic in the diet improved feed intake in broilers (REHMAN et al., 2020). Many factors affect efficiency of probiotics including species and strain of selected microorganism, probiotic preparation method – sensory attributes of carrier materials may play a distinct role in feed acceptance and intake –, survival of microorganisms in the gastrointestinal tract, the environment for raising broilers, nutritional management (including probiotic application time and route), physical and immunologic status of the animals, the lineage of birds evaluated, as well as age and lack of association with mother hens and concomitant use of antibiotics (HASHIM et al., 2021).

Because in this trial water and feed intakes were not or only marginally affected by dietary treatments, it is not surprising that the water to feed intake ratio across diets in this trial also was uniform. However, water to feed intake ratios in the starter phase were higher than that of other rearing phases and also, in the finisher phase the ratios were lower compared to the two other rearing phases. It has been stated that chicks with lower capacity of gastrointestinal tract consume less feed (ESMAIL, 2013). In this case, lower feed intake in the first week of age in chickens may be due to incomplete development of gastrointestinal tract and consequently higher water to feed intake ratio in this period of age.

5 Conclusion

Inclusion of various additives administered through drinking water had no significant effects on daily water intake in broiler chicken. Moreover, water to feed intake ratio as a tool for measurement of adequate water intake in broilers was not significantly influenced by additives. Hence the administration with drinking water may be a viable way to supply oregano oil or a probiotic *Enterococcus faecium* strain, or both, to diets of young, rapidly growing chicken without compromising their acceptance to consume water. Further studies are needed in order to elucidate the effects of different levels of these feed additives on water and feed intake in broilers.

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References

ADASZYŃSKA-SKWIRZYŃSKA, M. and D. SZCZERBIŃSKA (2019): The effect of lavender (*Lavan- dula angustifolia*) essential oil as a drinking water supplement on the production performance, blood biochemical parameters, and ileal microflora in broiler chickens. Poult. Sci. **98,** 358–365.

AGUILAR, C.A.L., K.R.D.S. LIMA, M.C. MANNO, F.B. TAVARES, V.P.D. SOUZA and D.L. FERNANDES NETO (2013): Effect of copaiba essential oil on broiler chickens' performance. Acta Sci. Anim. Sci. **35**, 145–151.

ALLOUI, N.M, A. AGABOU and N. ALLOUI (2014): Application of herbs and phytogenic feed additives in poultry production-A Review. Global J. Anim. Sci. Res. **2**, 234–243.

ALP, M., M. MIDILLI, N. KOCABAĞLI, H. YILMAZ, N. TURAN, A. GARGILI and N. ACAR (2012): The effects of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin G level, and oocyst count in broilers. J. Appl. Poult. Res. **21**, 630– 636.

AYALEW, H., H. ZHANG, J. WANG, S. WU, K. QIU, G. QI, A. TEKESTE, T. WASSIE and D. CHANIE (2022): Potential feed additives as antibiotic alternatives in broiler production. Front. Vet. Sci. **9**, 916473.

CAKIR, S., M. MIDILLI, M. ALP, H. YLIMAZ, O.H. MUGLAL, N. TURAN and N. KOCABAGLI (2008): Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. S. Afr. J. Anim. Sci. **38**, 21–27.

CAZARES-GALLEGOS, R., R. SILVA-VAZQUEZ, C.A. HERNANDEZ-MARTINEZ, J.G. GUTIERREZ-SOTO, J.R. KAWAS-GARZA, M.E. HUME and G.M. MENDEZ-ZAMORA (2019): Performance, carcass variables, and meat quality of broilers supplemented with dietary Mexican oregano oil. Braz. J. Poult. Sci. **21**, 1–10.

COLLETT, S.R. (2012): Nutrition and wet litter problems in poultry. Anim. Feed Sci. Technol. **173**, 65–75.

DENLI, M. and R. DEMIREL (2018): Replacement of antibiotics in poultry diets. CABI Rev. **13**, 35.

DOBROWOLSKI, P., E. TOMASZEWSKA, R. KLEBANIUK, A. TOMCZYK-WARUNEK, S. SZYMANCZYK, J. DONALDSON, I. ŚWIETLICKA, M. MIELNIK-BLASZCZAK, D. KUC and S. MUSZYNSKI (2019): Structural changes in the small intestine of female turkeys receiving a probiotic preparation are dose and region dependent. Animal **13**, 2773–2781.

DUNCAN, D.B. (1955): Multiple range and multiple F-tests. Biometrics **11**, 1–42.

ESCALERA-VALENTE, F., J.L. LOYA-OLGUÍN, C.A. CARMONA-GASCA, S. MARTÍNEZ-GONZÁLEZ and F. ÁVILA-RAMOS (2016): Effect of oregano oil supplementation in diets formed using either crude soybean oil or acidulated soybean oil soap stock as source of energy on the growth performance parameters of broilers. Arch. Med. Vet. **48**, 209–214.

ESMAIL, S.H. (2013): Factors affecting feed intake of chickens. World Poult. **29**, 15–17. https://www.poultryworld.net/health-nutrition/factors-affecting-feed-intake-of-chick- ens/.

FAIRCHILD, B.D. and C.W. RITZ (2015): Poultry drinking water primer. Bulletin 1301, UGA extension. Univ. Georgia, Athens, GA, USA. https://hdl.handle.net/10724/12452.

FRANKIC, T., M. VOLJC, J. SALOBIR and V. REZAR (2009): Use of herbs and spices and their extracts in animal nutrition. Acta Agric. Slov. **94**, 95–102.

HASHIM, Z., D.J. DAILIN., D.N.A. ZAIDEL, A.H.M. YUSOF, E. SALLEH, Y.M.M. JUSOH, N. PA'E, I.I. MUHAMAD, S. SELVAMANI, T. HO and H. EL ENSHASY (2021): Probiotics application in poultry industry: From feed to meat. Biosci. Res. **18,** 496–514.

HIDAYAT, M.N., R. MALAKA, L. AGUSTINA and W. PAKIDING (2018): Effect of *Lactobacillus* sp. probiotics on intestinal histology, *Escherichia coli* in excreta and broiler performance. J. Indonesian Trop. Anim. Agric. **43**, 445–452.

JAFARI, R.A., A. FAZLARA and M. GOVAHI (2006): An investigation into Salmonella and fecal coliform contamination of drinking water in broiler farms in Iran. Int. J. Poult. Sci. **5,** 491–493.

KAMPHUES, J., G. FLACHOWKSY, H. RIEGER and U. MEYER (2019): Für und wider eine Verabreichung von Futtermittezusatzstoffen über das Tränkwasser. Übers. Tierernährg. **43**, 205–248.

KARIMI TORSHIZI, M.A., A.R. MOGHADDAM, S.H. RAHIMI and N. MOJGANI (2010): Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. Br. Poult. Sci. **51**, 178–184.

KHOSRAVINIA, H. (2015): Effects of *Satureja khuzistanica* essential oils in drinking water on mortality, production performance, water intake, and organ weights in broiler chickens reared under heat stress condition. Int. J. Biometeorol. **59**, 1711–1719.

KOGUT, M.H. (2013): The gut microbiota and host innate immunity: regulators of host metabolism and metabolic diseases in poultry? J. Appl. Poult. Res. **22**, 637–646.

LEE, K.W., H. EVERTS, H.J. KAPPERT, M. FREHNER, R. LOSA and A.C. BEYNEN (2003): Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br. Poult. Sci. **44**, 450–457.

LEESON, S. and J.D. SUMMERS (2005): Commercial Poultry Nutrition, 3rd Edition. Nottingham Univ. Press, Nottingham, UK, p. 398.

MARKS, H.L. and G.M. PESTI (1984): The roles of protein level and diet form in water consumption and abdominal fat pad deposition of broilers. Poult. Sci. **63**, 1617–1625.

MCCREERY, D.H. (2015): Water Consumption Behavior in Broilers. Graduate Theses and Dissertations. Retrieved from https://scholarworks.uark.edu/etd/1301.

MOUNTZOURIS, K.C., P. TSIRTSIKOS, E. KALAMARA, S. NITSCH, G. SCHATZMAYR and K. FEGEROS (2007): Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult. Sci. **86**, 309–317.

MURUGESAN, G.R, B. SYED, S. HALDAR and C. PENDER (2015): Phytogenic feed additives as an alternative to antibiotic growth promoters in broiler chickens. Front. Vet. Sci. **2,** 1–6.

OLNOOD, C.G, S.S.M. BESKI, M. CHOCT and P.A. IJI (2015): Novel probiotics: Their effects on growth performance, gut development, microbial community and activity of broiler chickens. Anim. Nutr. **1**, 184–191.

PESTI, G.M., S.C. AMATO and L.R. MINEAR (1985): Water consumption of broiler chickens under commercial conditions. Poult. Sci. **64**, 803–808.

RAVINDRAN, V. (2014): Nutrition of meat animals. Poultry. In: Dikeman M, C. Devine (Eds.) Encyclopedia of Meat Sciences, 2nd ed. Academic Press, Oxford, UK, pp. 463470.

REHMAN, A., M. ARIF, N. SAJJAD, M.Q. AL-GHADI, M. ALAGAWANY, M.E. ABD EL-HACK, A.R. ALHIMAIDI, S.S. ELNESR, B.O. ALMUTAIRI, R.A. AMRAN, E.O.S. HUSSEIN and A.A. SWELUM (2020): Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity. Poult. Sci. **99**, 6946–6953.

ROURA, E., M.W. BALDWIN and K.C. KLASING (2013): The avian taste system: Potential implications in poultry nutrition. Anim. Feed Sci. Technol. **180**, 1–9.

SHEWITA, R.S., M. EL-KATCHA, M.A. SOLTAN and M.E. SEDEIK (2016): Impact of using *Enterococcus faecium* as a probiotic in broiler diets. Alex. J. Vet. Sci. **51**, 102–113.

SILVA-VÁZQUEZ, R., L. MELÉNDEZ, E. SANTELLANO-ESTRADA, C. RODRIGUEZ-MUELA, G. VILLALOBOS, G. MENDEZ-ZAMORA and M. HUME (2015): Performance of broiler chickens supplemented with Mexican oregano oil (*Lippia berlandieri* Schauer). Rev. Bras. Zootec. **44**, 283–289.

TEKELI, A., L. CELIK, H.R. KUTLU and M. GORGULU (2006): Effect of dietary supplemental plant extracts on performance, carcass characteristics, digestive system development, in testinal microflora and some blood parameters of broiler chicks. In: XII. Europ. Poult. Conf. Verona, Italy, 6 pp. https://www.cabi.org/Uploads/animal-science/worlds-poultry-science-association/WPSA-italy-2006/10283.pdf.

THOMKE, S. and K. ELWINGER (1998): Growth promotants in feeding pigs and poultry. III. Alternatives to antibiotic growth promotants. Ann. Zootech. **47**, 245–271.

VAN DER KLIS, J.D. and L. DE LANGE (2013): Water intake in poultry. In: Proc. 19th Europ. Symp. Poult. Nutr., Potsdam, Germany, pp. 102–107.

VDLUFA [Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten] (2012): Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik VDLUFA Methodenbuch), Bd. III. Die Chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

VO, K.V., M.A. BOONE and W.E. JOHNSTON (1978): Effect of three lifetime ambient temperatures on growth, feed and water consumption and various blood components in male and female Leghorn chickens. Poult. Sci. **57**, 798–803.

WENK, C. (2003): Herbs and botanicals as feed additives in monogastric animals. Asian- Aust. J. Anim. Sci. **16**, 282–289.

WINDISCH, W., K. SCHEDLE, C. PLITZNER and A. KROISMAYR (2008): Use of phytogenic products as feed additives for swine and poultry. J. Anim.Sci. **86,** E140-E148.

WPSA (1984): World's Poultry Science Association. Working Group No. 2 – Nutrition: the prediction of apparent metabolizable energy values for poultry in compound feeds. World's Poult. Sci. J. **40**, 181–182.

YU, L.C.H., J.T. WANG, S.C. WEI and Y.H. NI (2012): Host-microbial interactions and regulation of intestinal epithelial barrier function: From physiology to pathology. World J. Gastrointest. Pathophysiol. **3**, 27 –43.

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The effect of inclusion in drinking water, either singly or alternating, of oregano oil and a probiotic *Enterococcus faecium* strain on performance of growing broiler chickens

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Summary

The hypothesis of this study was that the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would improve growth performance of broiler chickens during a 42-day growth period. One-day-old Ross 308 chicks (mixed sex; mean body weight 45.1 g (standard deviation 1.04 g) were randomly assigned to four experimental groups at the start of the experiment: control (without supplement), probiotic (continuous supply of an *E. faecium* commodity [minimum activity per kg: 3.3×10^{12} colony forming units] with drinking water at 200 mg/L), oregano oil (75,000 mg/kg of product; first three days of each week at 0.2 mL/L drinking water), and oregano oil-probiotic (addition of oregano oil (0.2 mL/L) for three days and *E. faecium* commodity (200 mg/L) for four days to drinking water), each group with 10 replicates and each replicate with 10 chicks. Intakes of water and feed and body weights were determined. Body weight gain and feed conversion ratio were calculated to determine overall performance. Moreover, weights of liver and abdominal fat pad were assessed. Different additives supplied with drinking water did not affect ($P > 0.05$) performance of broiler chickens or liver and abdominal fat pad weights. Future studies should be conducted applying varying concentrations of the additives supplied with drinking water.

Keywords: broiler, drinking water, growth performance, oregano, probiotic

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Zusammenfassung

Effekte einer Verabreichung von Oreganoöl und eines probiotischen *Enterococcus faecium*-Stammes im Tränkwasser – einzeln oder alternierend – auf die Wachstumsleistung von Masthühnern.

Die Hypothese der Studie war, dass die Verabreichung von Oreganoöl und einem probiotischen *Enterococcus faecium*-Stamm im Tränkwasser, entweder einzeln oder alternierend, sich günstig auf die Wachstumsleistung von Masthühnern während einer 42-tägigen Mastperiode auswirkt. Gemischtgeschlechtliche Küken der Herkunft Ross 308 (mittlere Lebendmasse 45.1 g (Standardabweichung 1.04 g) wurden zu Versuchsbeginn zufällig auf vier Versuchsgruppen verteilt: Kontrolle (ohne Futterzusatzstoff im Tränkwasser), Probiotikum (kontinuierliche Gabe eines *E. faecium*-Produkts [Mindestaktivität pro kg: 3.3 × 10^{12} koloniebildende Einheiten] im Tränkwasser [200 mg/L]), Oreganoöl (75,000 mg/kg Produkt; jeweils an den ersten drei Tagen jeder Woche [0.2 mL/L Tränkwasser]) und Oreganoöl-Probiotikum (Zu gabe über das Tränkwasser von Oreganoöl [0.2 mL/L] über drei Tage und das *E. faecium* Produkt [200 mg/L] über 4 Tage/Woche). Jede Versuchsgruppe wurde in 10-facher Wieder holung geprüft und jede Wiederholung bestand aus 10 küken. Die Wasser- und Futterauf nahmen sowie Körpermassen wurden über 42 Tage quantifiziert. Die Körpermassezunah men und der Futteraufwand wurdenals Leistungsmerkmale ermittelt. Zusätzlich wurden Leber- und Abdominalfettpolstermassen ermittelt. Die Wachstumsleistung der Masthühner sowie Leber- und Abdominalfettpolstermassen wurden nicht durch die Zulagen im Tränk- wasser beeinflusst (P > 0.05). In weiteren Studien sollte untersucht werden, wie sich die Verabreichung unterschiedlicher Konzentrationen der geprüften Zusatzstoffe im Tränkwasser auf das Leistungsgeschehen auswirkt.

Schlüsselwörter: Masthühner, Tränkwasser, Wachstumsleistung, Oregano, Probiotikum

1 Introduction

Feed additives of various categories for farm animals are typically administered with dry or, sometimes, wet feeds (FORBES, 2003; SCHEDLE, 2016). To come into effect in the digestive tract of animals, feed additives need sufficient volumes of aqueous solutions like saliva, gastric or intestinal juices. As feed is chewed, e.g. in ruminants and pigs, particles are reduced in size and saliva is secreted to lubricate the bolus and enable swallowing. These proces ses have been most intensively studied in cattle and systematically reviewed by, e.g., BEAUCHEMIN (2018). Compared with ruminant and non-ruminant mammals, chickens and other avian species are distinctly different as they do not chew their food in the mouth and, therefore, saliva flow is low (7 – 30 mL/d in chickens; compiled by KLASING, 1998 and RODRIGUES and CHOCT, 2018) and plays a minor role in body water flow. This may result in delayed effectiveness of feed additives, which may further be hampered by the rapid digesta passage through the gastrointestinal tract of chicken. Based on these considerations, it appears remarkable that only little attention has been given to studies on administration of feed additives to poultry with drinking water.

Recently, we have observed that the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, did not negatively affect feed and water intake of broiler chickens during a 42-day growth period (IZADI et al., 2025). Therefore, in the same experiment, performance was measured and hypothesised that feed additives supplied via drinking water are an efficient way to support and improve performance without negative side effects.

2 Material and Methods

2.1 Experimental design and broiler management

Four hundred one-day-old Ross 308 chicks (mixed sex) were purchased from a commercial hatchery. The initial body weight (BW) of each chick was determined at the beginning of the experiment (average (with standard deviation) 45 g (1.04 g). The birds were randomly assigned to four dietary treatment groups, namely control, probiotic, oregano oil, and oregano oil-probiotic, each with 10 replicates and each replicate with 10 chicks. The birds were reared in a room from d 0 to 42, housed in pens of the same size, 110 cm × 120 cm for 10 birds. The pen floors were covered with wood shaving litter with a thickness of 5 cm at the beginning of the trial. Dry litter was added as necessary to maintain a low moisture level of the litter and lower the risk for foot-pad dermatitis. The pens were equipped with one bucket nipple drinker (10-L capacity) and one hanging plastic feeder. All chicken had *ad libitum* access to drinking water and feed during the 42-day trial. Moreover, temperature, lighting, and vaccination programs (Newcastle disease) were performed on the research station during the rearing phase according to standard recommendations. The experimental protocol was performed following the ARRIVE guidelines (https://arriveguidelines.org/) and were approved by the responsible Animal Care Committee.

2.2 Experimental treatments

A crumbled starter diet was provided to the birds from day 0 to 7, followed by a pelleted grower diet from day 8 to 14, and a pelleted finisher diet from day 15 to 42. The chemical composition of the diets was analysed in accordance with the methods of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2012) and encompassed dry matter, crude fat, crude fibre, crude protein, and ash. In addition, starch, sugar, calcium, sodium, potassium, and phosphorus concentrations were also determined (VDLUFA, 2012).

The N-corrected apparent metabolizable energy (ME) content was calculated from chemical composition according to WPSA (1984). Data of ingredients and chemical composition of the basal diet are presented in Table 1.

2.3 Sources of probiotic and oregano oil additives

The probiotic used in this study was *E. faecium* (DSM 7134, minimum activity per kg: 3.3×10^{12} colony forming units; Lovit Probiotic; Lohmann Animal Nutrition, Cuxhaven Germany) as water-soluble supplement. This supplement was incorporated constantly into drinking water of the probiotic group at 200 mg/L. Oregano oil (75,000 mg/kg; Lovit Progano Liquid; Kaesler Animal Nutrition, Cuxhaven, Germany) at level of 0.2 mL/L was incorporated into drinking water of the oregano oil group for the first three days per week. The combined treatment group, oregano oil-probiotic, received oregano oil in their drinking water for the first three days and the probiotic the other four days per week. Additives were mixed with drinking water based on calculated drinking water usage (mL) per day. The water mixtures were prepared shortly before they were offered to the birds in the nipple drinkers for each pen.

2.4 Feed intake and feed conversion ratio

Feed intake of broilers was measured for starter, grower, and finisher diets on a per pen basis. The offered feed for each pen was weighed separately and recorded at the beginning of each week and then residual feed in the feeders in each pen was weighed at the end of each week at day 7, 14, 21, 28, 35 and 42 of the trial; these values were divided by the number of birds in each pen to yield average values per pen. The average quantity of the offered feed minus the average quantity of the residual feed at the end of each week resulted in the total consumption per week which was divided by seven to calculate the average daily feed intake per bird. Data in week 1 of the trial were considered as starter period, week 2 as grower period and weeks 3, 4, 5 and 6 were considered as finisher period.

As an indication of the general health status, daily mortality rate was determined in this way that all diseased and dead birds were removed from the pen, weighed, marked with a description and recorded. Records of removed birds were needed for an accurate calculation of feed intake which were corrected for mortality records.

Feed conversion ratio (FCR) was calculated as the ratio of feed consumed (g/day) to daily BW gain (BWG) in the same period.

Tab. 1. Ingredients and chemical composition of the diets
Bestandteile und chemische Zusammensetzung der Rationen

	Periods of age		
	Starter (days 0 – 7)	Grower (days 8 – 14)	Finisher (days 15 – 42)
	Ingredients (decreasing order of their proportions)		
	Wheat	Wheat	Wheat
	Corn	Corn	Corn
	Soybean meal	Soybean meal	Soybean meal
	Soybean	Soybean	-
	Rapeseed meal	Rapeseed meal	Rapeseed meal
	Soybean oil	Soybean oil	Soybean oil
	Calcium carbonate	Calcium carbonate	Calcium carbonate
	Mono-calcium phosphate	Mono-calcium phosphate	-
	Sodium carbonate	Sodium carbonate	-
	-	Lignocellulose	Lignocellulose
	Chemical composition (g/kg unless stated)		
ME (MJ/kg)	12.1	12.4	13.3
Dry matter	919	910	912
Crude protein	213	205	175
Crude fat	45.0	58.0	82.0
Crude fibre	55.4	43.5	44.0
Ash	57.2	50.2	38.0
Sugar	33.9	31.4	34.1
Starch	408	407	439
Calcium	10.8	7.00	5.30
Sodium	1.10	1.20	1.10
Potassium	9.50	8.90	7.00
Phosphorus	6.44	5.67	4.57

Abbreviations: ME, N-corrected apparent metabolizable energy (calculated according to WPSA, 1984).

2.5 Sampling and measurement of liver and abdominal fat pad weights

One bird in each pen – as close as possible to the average BW of pen mates irrespective of sex – was selected and weighed with all pen mates after 5 h of starving at day 42. This weight was referred to as final live BW. Chickens were then slaughtered by cervical dislocation and liver and abdominal fat pad samples were collected as follows. The collection of abdominal fat pad included leaf fat surrounding the cloaca and abdominal muscles except fat surrounding the gizzard, and the fat pad weight (g) was determined directly after sampling. The liver was separated from the carcass and weighed (g). The weight of livers and fat tissues were also divided by the final BW of chickens before slaughtering (pen-based) and expressed as percentage of final BW:

Relative weight (%) of liver or abdominal fat pad =
[(liver or abdominal fat pad weight (g))/ (final BW)] × 100.

2.6 Statistical analysis

The experimental data were statistically analysed as a completely randomized design using SPSS Software (v 19.0; SPSS Inc, Chicago, IL, USA). Values were checked for normality before conducting an analysis of variance (ANOVA). Data of performance variables were subjected to a one-way ANOVA and comparison between treatment (i.e., diet) means was conducted using Duncan's multiple range test (DUNCAN, 1955). The nonparametric Kruskal–Wallis test was applied to evaluate treatment effects on fat pad and liver weights. Correlation coefficients between final BW and fat pad and liver weights, respectively, were obtained from Pearson's correlations in a 2-tailed test. Moreover, a scatter plot was generated to determine the relationship between final BW and liver weight. P-values < 0.05 were considered as indicating significant differences. A trend was observed when P-values were ≥ 0.05 but < 0.10.

3 Results

The values of performance parameters are shown in Table 2. The broilers of all dietary treatment groups consumed their feed uniformly throughout the starter, grower and finisher periods without any indication of differences between dietary treatment means. The uniform feed intake was paralleled by a consistent BW and BWG across the three periods for all four dietary treatment groups. Consequently, also the FCR was not different among the treatments. In the starter and grower periods, the FCR was close to 1.0 and it increased to approximately 1.4 in the finisher period.

Tab. 2. Mean values of performance parameters of broiler chickens in response to different additives supplied with drinking water
Mittelwerte der Leistungsmerkmale von Masthühnern bei Verabreichung ausgewählter Zusatzstoffe im Tränkwasser

	Dietary treatment groups					
Parameters	Control	Probiotic	Oregano oil	Oregano oil-probiotic	SEM	*P*-value
Feed intake (g/d; from IZADI et al., 2025)						
d 0–7	22.0	22.5	21.8	22.0	0.140	0.322
d 8–14	45.0	45.4	43.6	43.8	0.466	0.485
d 15–42	130	129	128	131	0.788	0.493
Body weight (g)						
d 0–7	197	202	195	197	1.09	0.178
d 8–14	503	508	494	499	3.30	0.521
d 15–42	3085	3032	3033	3081	19.39	0.656
Average daily gain (g/d)						
d 0–7	21.6	22.3	21.4	21.8	0.15	0.227
d 8–14	43.6	43.7	42.6	43.2	0.36	0.731
d 15–42	92.2	90.2	90.7	92.2	0.65	0.594
Feed conversion ratio (g/g)						
d 0–7	1.02	1.01	1.02	1.01	0.003	0.352
d 8–14	1.03	1.04	1.02	1.01	0.007	0.634
d 15–42	1.42	1.43	1.42	1.43	0.006	0.748

Abbreviation: SEM, standard error of the means.

In accordance with the performance parameters presented in Table 2, data shown in Table 3 indicate that broilers in the four dietary treatment groups had also very similar relative (% of BW) and absolute (g) weights of liver and abdominal fat pads. Because the liver plays a key role in lipid metabolism, this data demonstrate that the additives supplied with drinking water did not exert any observable adverse effect but, also no beneficial effect, on body fat metabolism and accretion.

Tab. 3. Final live body weight and weights of liver and abdominal fat pad of broiler chickens in response to different additives supplied with drinking water.
Körpermasse zur Schlachtung sowie Leber- und Abdominalfettpolstermassen von Masthühnern bei Verabreichung ausgewählter Zusatzstoffe im Tränkwasser

Parameters	Dietary treatment groups					
	Control	Probiotic	Oregano oil	Oregano oil-probiotic	SEM	*P*-value
Final live body weight (g)	3001	3051	2871	3114	54.0	0.447
Liver weight (g)	58.7	60.2	55.7	63.5	1.81	0.503
Abdominal fat pad weight (g)	45.3	42.3	46.2	46.0	1.86	0.875
Liver weight (% of BW)	1.95	1.97	1.94	2.03	0.041	0.884
Abdominal fat pad weight (% of BW)	1.52	1.37	1.60	1.48	0.056	0.538

Abbreviations: BW, body weight; SEM, standard error of the means.

Furthermore, the relationship between final live BW of broilers and liver weight was positive and significant ($P < 0.01$; Fig. 1). It is also evident from Figure 1 that for the heavier birds the liver weights varied much more than for the lighter animals. Birds weighing not more than 3,000 g at slaughter had very similar liver weights whereas for the heavier birds the liver weights at the same BW sometimes differed considerably such that the lighter livers had less than two-thirds of the weight of the heaviest livers.

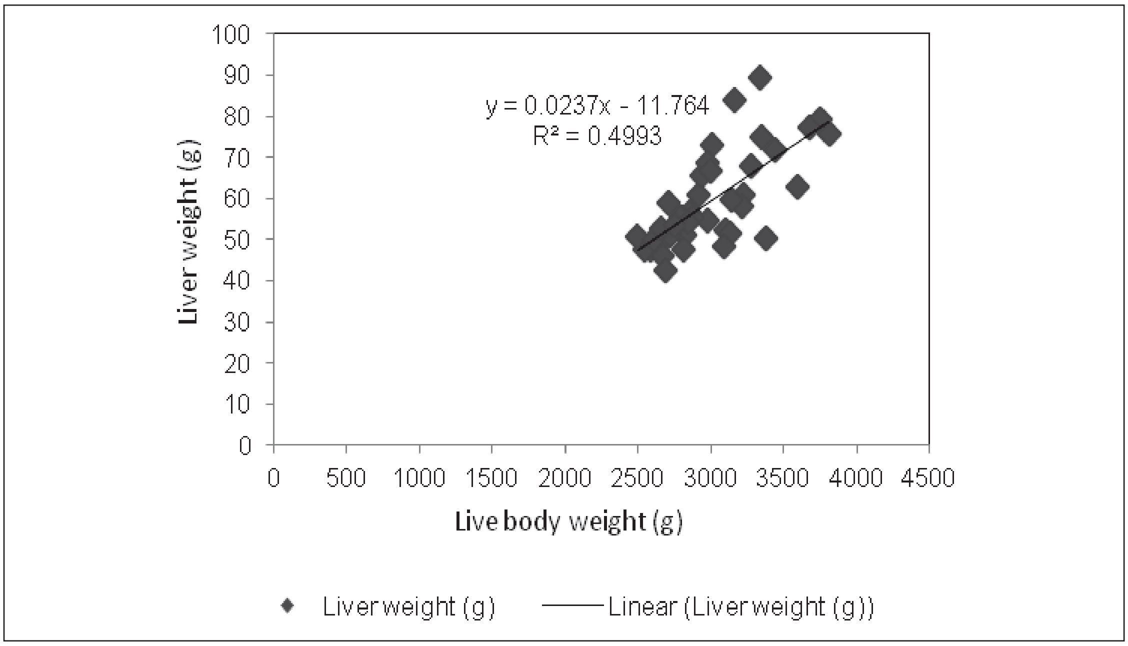

Fig. 1. Relationship between final live body weight (g) and liver weight (g) of broiler chickens supplied with different additives in drinking water.
Beziehung zwischen der Körpermasse zur Schlachtung und der Lebermasse von Masthüh- nern bei Verabreichung ausgewählter Zusatzstoffe im Tränkwasser.

4 Discussion

A common nutritional strategy to increase farm animal production and health and reduce side effects of antibiotics is to utilize phytogenic substances as natural feed additives and microbial preparations as probiotics that can help to improve performance and promote optimal health of food animals (GADDE et al., 2017). In our study, the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, on growth performance of broiler chickens was investigated.

TIMMERMAN et al. (2006) studied mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics and observed improved productivity based on an index taking into account daily weight gain, feed efficiency and mortality. KARIMI TORSHIZI et al. (2010) compared two routes of administration, namely feed and water, of a probiotic preparation consisting of 9 different microorganisms. They reported that performance of broilers in terms of BWG, feed intake and FCR improved when probiotic was provided via drinking water, compared to the control and feed groups. The authors assumed that the improved performance was induced by an enhanced immune modulation.

Similarly, ECKERT et al. (2010) compared probiotic administration to broilers via feed (post-pelleting spraying) and via drinking water and observed that BWG and FCR ratio were improved when the probiotic was administered via drinking water. Similarly, inclusion of *Enterococcus faecium* in drinking water has improved performance of broilers (CHAVEZ et al., 2016).

Recently, RAHAYU et al. (2023) compared effects of herbs supplemented to either feed or drinking water on biochemical blood characteristics of broilers and found inconsistent effects which, at least in part, may be related to the use of a diverse herb mixture consisting of eight different herbal species. Comparing studies that utilized different herbal ingredients is extremely difficult because the effectiveness of these studies will be additionally influenced by various factors like plant composition, supplementation levels, method and prevalence of application, animal production stage, and environmental stress factors. Furthermore, it is still unclear if certain individual effects are due to a single component or reflect a synergistic effect of multiple components (HIPPENSTIEL et al., 2011). Thus, determining the chemical composition of the used herbal substances is essential for identifying their impact and consequently, optimal composition.

The EFSA Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP, 2010) has published a "Statement on the use of feed additives authorised/applied for use in feed" and concluded that "there is no need to separately assess safety and efficacy of an additive administered via water […], provided that the exposure of the animals is essentially the same". More recently, KAMPHUES et al. (2019) have systematically and comprehensively evaluated pros and cons of supplementation of feed additives to drinking water and have also noted concerns that distinct additives may change water properties such as nutrient concentrations, sensory attributes and microbial status, any or all of which could negatively impact on voluntary water consumption. These hints should always be considered before feed additives are provided with drinking water.

5 Conclusion

Inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, had no effects on growth performance, FCR and on liver and abdominal fat pad weights. Although no beneficial responses of broilers were observed to the supply of additives with drinking water, the absence of adverse effects, shows, for the investigated types of additives, the general applicability of the route of administration, i.e., supply in drinking water and not in feed. Therefore, it appears justified to conduct further studies in order to elucidate the effects of different levels of these feed additives on broiler performance when supplied in drinking water. Future studies should also consider conventional watering systems.

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References

BEAUCHEMIN, K.A. (2018): *Invited review*: Current perspectives on eating and rumination activity in dairy cows. J. Dairy Sci. **101,** 4762-4784.

CHAVEZ, L.A., A. LÓPEZ and J.E. PAR (2016): Usage of *Enterococcus faecium* to improve productive parameters in broilers. Rev. Fac. Med. Vet. Zoot. **63**, 113–123.

DUNCAN, D.B. (1955): Multiple range and multiple F-tests. Biometrics **11**, 1–42.

ECKERT, N.H., J.T. LEE, D. HYATT, S.M. STEVENS, S. ANDERSON, P.N. ANDERSON, R. BELTRAN, G. SCHATZMAYR, M. MOHNL and D.J. CALDWELL (2010): Influence of probiotic administration by feed or water on growth parameters of broilers reared on medicated and non- medicated diets. J. Appl. Poult. Res. **19**, 59–67.

EFSA Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP) (2010): Scientific Opinion. Statement on the use of feed additives authorised/applied for use in feed when supplied via water. EFSA J. **8**(12):1956.

FORBES, J.M. (2003): Wet foods for poultry. Avian Poult. Biol. Rev. **14**, 175–193.

GADDE, U., W.H. KIM, S.T. OH and H.S. LILLEHOJ (2017): Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Health Res. Rev. **18**, 26–45.

HIPPENSTIEL, F., A.A.A. ABDEL-WARETH, S. KEHRAUS and K.-H. SÜDEKUM (2011): Effects of selected herbs and essential oils, and their active components on feed intake and performance of broilers – a review. Arch. Geflügelk. **75**, 226–234.

IZADI, M., H.-J. ALERT and K.-H. SÜDEKUM (2025): The effect of inclusion in drinking water, either singly or alternating, of oregano oil and a probiotic *Enterococcus faecium* strain on feed and water intake of growing broiler chickens. Züchtungsk. **97**, 123–135.

KAMPHUES, J., G. FLACHOWSKY, H. RIEGER and U. MEYER (2019): Für und wider eine Ver- abreichung von Futtermittezusatzstoffen über das Tränkwasser. Übers. Tierernährg. **43**, 205–248.

KARIMI TORSHIZI, M.A., A.R. MOGHADDAM, S.H. RAHIMI and N. MOJGANI (2010): Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. Br. Poult. Sci. **51**, 178–184.

KLASING, K.C. (1998): Comparative Avian Nutrition. CAB International, Wallingford, Oxon, UK, p. 18.

RAHAYU, I.D., A. MAHMUD, W. WIDODO, A. SUTANTO, A.D. ANGGRAINI, D.D. SISKAWARDANI, W. NURCAHYO and T. UNTARI (2023): The effects of herbs as feed additives through feed and drinking water on broiler blood parameters. Adv. Anim. Vet. Sci. **11**, 1524–1531.

RODRIGUES, I. and M. CHOCT (2018): Non-invited review. The foregut and its manipulation via feeding practices in the chicken. Poult. Sci. **97**, 3188–3206.

SCHEDLE, K. (2016): Sustainable pig and poultry nutrition by improvement of nutrient utilization – A review. Bodenkultur: J. Land Manage. Food Environ. **67**, 45–60.

TIMMERMAN, H.M., A. VELDMAN, E. VAN DEN ELSEN, F.M. ROMBOUTS and A.C. BEYNEN (2006): Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. Poult. Sci. **85**, 1383–1388.

VDLUFA [Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten] (2012): Handbuch der

Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA Methodenbuch), Bd. III. Die Chemische Untersuchung von Futtermitteln. VDLUFA- Verlag, Darmstadt, Germany.

WPSA (1984): World's Poultry Science Association. Working Group No. 2 – Nutrition: the prediction of apparent metabolizable energy values for poultry in compound feeds. World's Poult. Sci J. **40**, 181–182.

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The effect of inclusion in drinking water, either singly or alternating, of oregano oil and a probiotic *Enterococcus faecium* strain on gut health characteristics of growing broiler chickens

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Summary

The hypothesis of this study was that, during a 42-day growth period, the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would positively impact on gut health characteristics of growing broiler chickens such as excreta pH and dry matter content, and selected histomorphological parameters, i.e, villus height, crypt depth, villus height to crypt depth ratio, and crypt width. Four-hundred Ross 308 chicks (mixed-sex; mean body weight 45.1 g (standard deviation 1.04 g)) were randomly assigned to four experimental groups during the 42-day growth period: control (without supplement), probiotic (continuous supply of an *E. faecium* commodity [minimum activity per kg: 3.3×10^{12} colony forming units] with drinking water at 200 mg/L), oregano oil (75,000 mg/kg of product; first three days of each week at 0.2 mL/L drinking water), and oregano oil-probiotic (addition of oregano oil (0.2 mL/L) for three days and E. faecium commodity (200 mg/L) for four days to drinking water), each group with 10 replicates and each replicate with 10 broilers. Excreta pH and dry matter content were unaffected by dietary treatment. Only in the finisher phase of the growth period, the oregano oil group had slightly higher excreta dry matter content. However, the probiotic and oregano oil-probiotic supplements affected histomorphological parameters; the villus height in the ileum was higher compared with the other experimental groups. Moreover, in the ileum of broilers in the oregano oil-probiotic group, the crypt depth was deeper and the villus height to crypt depth ratio was wider than for the other experimental groups. Yet, crypt width in the ileum was not influenced by additives. Overall, the oregano oil-probiotic administration in drinking water positively affected selected histomorphological gut characteristics in growing broiler chickens. Consequently, the administration with drinking water may be a viable way to supply these types of feed additives to diets of growing broiler chickens. Future studies should be conducted applying varying concentrations of the additives supplied with drinking water.

Keywords: oregano oil, probiotic, *Enterococcus faecium*, broiler chickens

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Zusammenfassung

Effekte einer Verabreichung von Oreganoöl und eines probiotischen *Enterococcus faecium*-Stammes im Tränkwasser – einzeln oder alternierend– auf Merkmale der Darmgesundheit von Masthühnern.

Die Hypothese der Studie war, dass die Verabreichung von Oreganoöl und einem probiotischen *Enterococcus faecium*-Stamm im Tränkwasser, entweder einzeln oder alternierend, günstige Auswirkungen auf Merkmale der Darmgesundheit wachsender Masthühner während einer 42-tägigen Mastperiode hat. Gemischtgeschlechtliche Küken der Herkunft Ross 308 (mittlere Lebendmasse 45.1 g, Standardabweichung 1.04 g) wurden zu Versuchsbeginn zufällig auf vier Versuchsgruppen während der 42-tägigen Mastperiode verteilt: Kontrolle (ohne Futterzusatzstoff im Tränkwasser), Probiotikum (kontinuierliche Gabe eines *E. faecium*-Produkts [Mindestaktivität pro kg: 3.3×10^{12} koloniebildende Einheiten] im Tränkwasser [200 mg/L]), Oreganoöl (75,000 mg/kg Produkt; jeweils an den ersten drei Tagen jeder Woche [0.2 mL/L Tränkwasser]) und Oreganoöl-Probiotikum (Zugabe über das Tränkwasser von Oreganoöl [0.2 mL/L] über drei Tage und das *E. faecium*-Produkt [200 mg/L] über 4 Tage/Woche). Jede Versuchsgruppe wurde in 10-facher Wiederholung geprüft und jede Wiederholung bestand aus 10 Küken. Als Merkmale der Darmgesundheit wurden der pH-Wert der Exkreta und deren Trockenmassegehalt ermittelt sowie ausgewählte histomorphologische Parameter erfasst, namentlich Höhe der Darmzotten, Kryptentiefe, das Verhältnis dieser beiden Größen und die Kryptenbreite. Der pH-Wert der Exreta und ihr Trockenmassegehalt wurden nicht durch die Zulagen im Tränkwasser verändert. Die Zulage des Probiotikums und der Kombination Oreganoöl-Probiotikum bewirkte eine größere Höhe der Darmzotten im Ileum. Für die Kombination Oreganoöl-Probiotikum wurde auch eine ausgeprägtere Kryptentiefe ermittelt, die sich in einem weiteren Verhältnis von Zottenhöhe zu Kryptentiefe widerspiegelte. Insgesamt deuten diese Befunde auf eine günstige Beeinflussung der Darmgesundheit durch die geprüften Zulagen im Tränkwasser hin. In weiteren Studien sollte untersucht werden, wie sich die Verabreichung unterschiedlicher Konzentrationen der geprüften Zusatzstoffe im Tränkwasser auf die Darmgesundheit bei Masthühnern auswirkt.

Schlüsselwörter: Oreganoöl, Probiotika, *Enterococcus faecium*, Broiler

1 Introduction

The gut is described by SUGIHARTO (2016) as an essential organ system which plays an important role in feed digestion and host defence. Quantitatively, most of the digestion and absorption take place in the small intestine (TURK, 1982). In comparison to mammals, the gastrointestinal tract of chickens is noticeably shorter relative to the body length (YADAV and JHA, 2019). The small intestine acts as a natural habitat of numerous microbes, therefore, microbial balance in the intestine is an indicator of a healthy chicken (RIVENIA and ASYROFI, 2015). Moreover, maintenance of normal microarchitecture in the small intestine is essential for proper growth performance and development of broilers (SOHAIL et al., 2012). Due to the huge amount of feed handled by commercial poultry breeds, the digestive tract needs to function properly (SVIHUS, 2014). The gastrointestinal epithelium forms the boundary between the body and external environment. It effectively provides a selective permeable barrier that limits the permeation of luminal noxious organisms or molecules, such as pathogens, toxins, and antigens, while, at the same time, allowing the appropriate absorption of nutrients and water.

A proven strategy to support gut health was the inclusion of antibiotics in animal feeds which has been extensively used for decades in animal production systems, inclu sive poultry (HUYGHEBAERT et al., 2011). Poultry flocks are raised under intensive conditions, hence considerable quantities of in-feed antimicrobials have been applied to prevent diseases, and, by this means, have acted also as growth promoters (NHUNG et al., 2017). However, antimicrobial resistant poultry pathogens may contribute to occurrence of treatment failure resulting in economic losses and may generate a source of resistant bacterial genes including zoonotic bacteria (NHUNG et al., 2017). Antibiotic resistance among the bacterial pathogens as well as concerns over their widely use in animal feed has gained global interest in limiting antibiotic use in animal agriculture and its importance to find innovative antimicrobials that provide alternatives to conventional antibiotics (SEAL et al., 2013). Furthermore, the use of antibiotic feed additives has already been banned in many regions worldwide, e.g., in the European Union since January 1, 2006 (CASTANON, 2007).

Feed additives of plant origin, called phytogenic feed additives, phytobiotics or phytoadditives, are considered as alternative, non-antibiotic growth promoters, even though there are well established non-antibiotic growth promoters such as organic acids and probiotics (GUO et al., 2003). Phytogenic feed additives may adjust feed intake and stimulate digestive secretions leading to optimization of digestive capacity and risk reduction of digestive disorders (WENK, 2003). There is currently a great interest in essential oils from different herbs as feed additives in animal nutrition, as they have a greater biological activity than the raw material from which they were extracted (YITBAREK, 2015). Oregano (*Origanum vulgare*) oil is used in poultry industry as natural additive (AVILA RAMOS et al., 2017). It has been reported that the aromatic, slightly bitter taste and functional properties of oregano are related to the quantity and composition of its phenolic components (KARIMI et al., 2010). Major components in oregano are including carvacrol, thymol, γ-terpinene, and ρ-cymene (BURT, 2004). Oregano essential oils are known for their antimicrobial activity, as well as their antiviral and antifungal properties. In addition, these compounds have been reported to contain antioxidant, anti-inflammatory, antidiabetic activities and cancer inhibiting agents (LEYVA-LOPEZ et al., 2017).

Moreover, probiotics in the diet of poultry may improve growth performance, feed conversion efficiency, immune responses and act effectively against enteric pathogens (DHAMA et al., 2011). *Enterococcus faecium* belongs to the physiological intestinal flora of poultry and is used as a probiotic feed additive due to its effect on the intestinal microflora through the formation of lactate and short-chain fatty acids (YU et al., 2012). This bacterium competes with pathogenic microorganisms and its activity results in the selection advantage of beneficial intestinal bacteria (KOGUT, 2013).

Development of the small intestine as a critical digestive organ is essentially involved in nutrient digestion and absorption, therefore, its development is vital to achieve stable health and performance (KAWALILAK et al., 2010). The ileum as the most distal segment of the small intestine ends at the ileo-caeco-colic junction and although some digestion and absorption of fat, protein, and starch may take place, this segment is mainly thought to play a role as a site for water and mineral element absorption (SVIHUS, 2014). Small intestinal morphological parameters including villus height, crypt depth, and the villus height to crypt depth ratio are indicative of gut health in broiler chickens (HOSSAIN et al., 2015). Changes in the morphology of the gastrointestinal tract by adding appropriate supplements may give some information about its beneficial effects on the digestive tract (MURUGESAN et al., 2015). Probiotics have been reported to improve microbial balance in the gastrointestinal tract through bacterial antagonisms, competitive exclusion and immune stimulation (AWAD et al., 2008). Moreover, essential oil compounds such as thymol and carvacrol limit bacterial growth and modulate the pathogenicity of the bacteria in the gut (YIN et al., 2017). Therefore, it is assumed that a synergistic effect of oregano oil and a probiotic feed additive incorporated into drinking water may effectively improve gut health of broilers.

The hypothesis of this study was that the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would positively impact on gut health characteristics of growing broiler chicken. Consequently, this way of administration would be a viable alternative to supplying these types of feed additives to diets of young, rapidly growing chickens. Data from the same experiment on feed and water intake (IZADI et al., 2025a) have indicated that the administration with drinking water may be a viable way to supply oregano oil or a probiotic *E. faecium* strain, or both, to diets of growing broiler chickens without compromising their acceptance to consume water. Similarly, IZADI et al. (2025b) have reported that inclusion of oregano oil and a probiotic *E. faecium* strain in drinking water, either singly or alternating, had no effects on growth performance, feed conversion ratio and on liver and abdominal fat pad weights. Although no beneficial responses of broilers were observed to the supply of additives with drinking water, the absence of adverse effects, shows, for the investigated types of additives, the general applicability of the route of administration, i.e., supply in drinking water and not in feed.

2 Material and methods

2.1 Animals and diets

Four hundred one-day-old Ross 308 chicks (mixed sex) were purchased from a commercial hatchery. The initial body weight of each chick was determined at the beginning of the experiment (average, with standard deviation) 45.1 g, 1.05 g). The birds were randomly assigned to four dietary treatment groups, namely control, probiotic, oregano oil, and oregano oil-probiotic, each with 10 replicates and each replicate with 10 chicks. The birds were reared in a room from day 0 to 42, housed in pens of the same size, 110 cm × 120 cm for 10 birds. The pen floors were covered with wood shaving litter with a thickness of 5 cm at the beginning of the trial. Dry litter was added as necessary to maintain a low moisture level of the litter and lower the risk for foot-pad dermatitis. The pens were equipped with one bucket nipple drinker (10-L capacity) and one hanging plastic feeder. All chicken had *ad libitum* access to drinking water and feed during the 42-day trial. Moreover, temperature, lighting, and vaccination programs (Newcastle disease) were performed on the research station during the rearing phase following a protocol established at the research station. As an indication of the general health status, daily mortality rate was determined in this way that all diseased and dead birds were removed from the pen, weighed, marked with a description and recorded. Records of removed birds were needed for an accurate calculation of water and feed intake and growth characteristics which were corrected for mortality records.

A crumbled starter diet was provided to the birds from day 0 to 7, followed by a pelleted grower diet from day 8 to 14, and a pelleted finisher diet from day 15 to 42. The chemical composition of the diets was analysed in accordance with the methods of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2012) and encompassed dry matter, crude fat, crude fibre, crude protein, and crude ash. Moreover, nitrogen-free extract (NfE) was calculated subsequently. In addition, starch, sugar, calcium, sodium, potassium, and phosphorus concentrations were also determined (VDLUFA, 2012).

The N-corrected apparent metabolizable energy (ME) content was calculated from chemical composition according to WPSA (1984). Data of ingredients and chemical composition of the basal diet are presented in Table 1.

The probiotic used in this study was *E. faecium* (DSM 7134, minimum activity per kg: 3.3×10^{12} colony forming units; Lovit Probiotic; Lohmann Animal Nutrition, Cuxhaven Germany) as water-soluble supplement. This supplement was incorporated constantly into drinking water of the probiotic group at 200 mg/L. Oregano oil (75,000 mg/kg; Lovit Progano Liquid; Kaesler Animal Nutrition, Cuxhaven, Germany) at level of 0.2 mL/L was incorporated into drinking water of the oregano oil group for the first three days per week. The combined treatment group, oregano oil-probiotic, received oregano oil (0.2 mL/L) in their drinking water for the first three days and the *E. faecium* commodity (200 mg/L) the other four days per week. Additives were mixed with drinking water based on calculated drinking water usage (mL) per day. The water solutions were prepared shortly before they were offered to the birds in the nipple drinkers for each pen.

Tab 1. Ingredients and chemical composition of the diets
Bestandteile und chemische Zusammensetzung der Rationen

	Periods of age		
	Starter (days 0 - 7)	Grower (days 8 - 14)	Finisher (days 15 – 42)
Ingredients (decreasing order of their proportions)			
	Wheat	Wheat	Wheat
	Corn	Corn	Corn
	Soybean meal	Soybean meal	Soybean meal
	Soybean	Soybean	-
	Rapeseed meal	Rapeseed meal	Rapeseed meal
	Soybean oil	Soybean oil	Soybean oil
	Calcium carbonate	Calcium carbonate	Calcium carbonate
	Mono-calcium phosphate	Mono-calcium phosphate	-
	Sodium carbonate	Sodium carbonate	-
	-	Lignocellulose	Lignocellulose
Chemical composition (g/kg unless stated)			
ME (MJ/kg)	12.1	12.4	13.3
Dry matter	919	910	912
Crude protein	213	205	175
Crude fat	45.0	58.0	82.0
Crude fibre	55.4	43.5	44.0
Ash	57.2	50.2	38.0
Sugar	33.9	31.4	34.1
Starch	408	407	439
Calcium	10.8	7.00	5.30
Sodium	1.10	1.20	1.10
Potassium	9.50	8.90	7.00
Phosphorus	6.44	5.67	4.57

Abbreviations: ME, N-corrected apparent metabolizable energy (calculated according to WPSA, 1984).

2.2 Intestinal parameters

2.2.1 Sampling and histomorphological examination of the ileum

Chickens were slaughtered by cervical dislocation. For intestinal measurements, the small intestine of all broilers was carefully dissected. Medial parts (length approximately 2 cm) of the ileum between Meckel's diverticulum and the ileo-caecal junction were cut out and transferred to plastic bottles containing formalin solution; then they were refrigerated at 4°C until analysis. For histomorphological examination, the samples of the ileum segment of each bird were fixed in a 10% buffered formalin solution and gut tissues were embedded in paraffin wax blocks and then sections of 5 μm thickness were mounted onto glass slides and stained with Haematoxylin and Eosin. Morphometric analyses were done on 10 well-oriented and intact villi and 10 crypts. Criterion for villus selection was based on the presence of an intact Lamina propria (Prakatur et al., 2019). In addition, morphometric indices were villus height (distance between the tip of the villus to the crypt), crypt depth (distance between the base of the villus to the submucosa), and the crypt width measured at the midline of the crypt. Villus height, crypt depth, and crypt width were measured by using a microscope with image analyser and expressed as micrometers (μm). Also, villus height to crypt depth ratio was estimated subsequently by dividing villus height by crypt depth. For all parameters, average values for each bird were calculated.

2.2.2 Excreta sampling and analysis

The excreta collection (approximately 200 g) was conducted during the trial from each pen immediately after fresh droppings were excreted by an unknown number of birds per pen, at the end of each week at day 7, 14, 21, 28, 35, and 42. To prevent contamination of the excreta, any dropped feathers, feed particles or foreign materials were removed. Excreta samples of each group were divided into two parts. One part of the fresh excreta was used to analyse pH values and the other part was stored at −20°C until used for determination of excreta dry matter content. Week 1 was considered as starter phase, week 2 as grower phase and weeks 3 to 6 were considered as finisher phase of the growth period.

For measurement of the excreta dry matter content, samples were placed in the crucibles and weighed using a digital scale. The determination of the dry matter content was done in accordance with method 3.1 outlined in VDLUFA (2012).

A pH meter (SevenMulti, Mettler Toledo Schwerzenbach, Switzerland) was applied to measure the pH of excreta samples. The fresh excreta sample (1 g) was mixed with 20 mL of deionised water and homogenized. Then, the pH of each group's excreta was measured and the data was recorded.

2.3 Statistical analysis

All obtained raw data were entered into Microsoft Excel data base system to process the data with using the relevant statistical calculation module. Values were checked for normality assumption before the ANOVA. The data were analysed statistically in a completely randomized design and all data were subjected to one-way ANOVA for all variables using SPSS Software (v 19.0; SPSS Inc, Chicago, IL). Group means were compared using Duncan's multiple range test (Duncan, 1955). Differences between means with $p < 0.05$ were considered significant.

3 Results

The treatments had no effect on excreta pH of broilers throughout the 42-day growth period (Tab. 2). All pH values were > 7 for all experimental groups and were very homogenous among experimental groups within growth phase. The only noticeable exception from this general observation yet still not statistically different from the other experimental groups was the excreta pH value of the probiotic group in the grower phase.

Tab 2. Effects of supplements added to drinking water on excreta pH value of broiler chickens
Auswirkungen von mit dem Tränkwasser verabreichten Zusatzstoffen auf den pH-Wert der Exkremente der Masthühner

Growth periods	*Experimental groups*					
	Control	Probiotic	Oregano oil	Oregano oil-Probiotic	Pooled SEM	*P-value*
Starter phase (days 0-7)	7.73±0.218	7.70±0.244	7.61±0.206	7.64±0.301	0.038	0.671
Grower phase (days 8-14)	7.09±0.465	7.54±0.287	7.22±0.373	7.22±0.532	0.070	0.123
Finisher phase (days 15-42)	7.24±0.234	7.21±0.148	7.27±0.164	7.26±0.155	0.027	0.877

Note: Data are shown as means ± SD (standard deviation).
Abbreviation: SEM, standard error of means.

The supplements added to drinking water did not change ($P > 0.05$) excreta dry matter content during the 42-day growth period (Tab. 3). Moreover, the dry matter concentrations across growth phases and experimental groups ranged only from 22.0 to 25.4%, indicating that water flow and water exchange in the intestines were not affected by dietary supplements.

Tab 3. Effects of supplements added to drinking water on excreta dry matter content (%) of broiler chickens
Auswirkungen von mit dem Tränkwasser verabreichten Zusatzstoffen auf den Trockenmasse-Gehalt (%) der Exkremente der Masthühner

Growth periods	*Experimental groups*					
	Control	Probiotic	Oregano oil	Oregano oil-Probiotic	Pooled SEM	*P-value*
Starter phase (days 0-7)	24.0 ± 6.62	22.5 ± 4.04	23.6 ± 2.55	23.5 ± 2.07	0.645	0.886
Grower phase (days 8-14)	25.4 ± 7.27	22.1 ± 2.10	23.5 ± 4.75	24.0 ± 4.72	0.790	0.552
Finisher phase (days 15-42)	22.3 ± 1.11	22.0 ± 2.09	23.3 ± 1.89	22.9 ± 1.97	0.287	0.334

Note: Data are shown as means ± SD (standard deviation).
Abbreviation: SEM, standard error of means.

Other than excreta pH and dry matter content, histomorphological characteristics of the ileum segment responded differently to supplements added to drinking water (Tab. 4). Villus height was greater in the probiotic and oregano oil-probiotic groups than in the two other groups. The crypt depth was lower in the oregano oil-probiotic group compared to other experimental groups. The villus height to crypt depth ratio was wider in the oregano oil-probiotic group than in the other experimental groups. Crypt width was the only histomorphological parameter which was not affected by experimental treatments.

Tab 4. Effects of supplements added to drinking water on histomorphological parameters of the ileum segment of broiler chickens
Auswirkungen von mit dem Tränkwasser verabreichten Zusatzstoffen auf histomorphologische Parameter des Ileumsegments der Masthühner

Parameters	*Experimental groups*			
	Control	Probiotic	Oregano oil	Orgeano oil-Probiotic
Villus height (μm)	$535^{b*} \pm 14.9$	$590^{a} \pm 16.5$	$553^{b} \pm 27.4$	$569^{a} \pm 13.6$
Crypt depth (μm)	$151^{a} \pm 6.76$	$159^{a} \pm 4.35$	$154^{a} \pm 7.99$	$146^{b} \pm 10.3$
Villus height to crypt depth ratio	$3.55^{b} \pm 0.23$	$3.71^{b} \pm 0.14$	$3.60^{b} \pm 0.11$	$3.91^{a} \pm 0.26$
Crypt width (μm)	95.1 ± 1.68	95.4 ± 3.32	95.7 ± 2.52	95.2 ± 3.61

Note: Data are shown as means ± SD (standard deviation).
*Different letters within a row indicate significant differences between experimental groups at (P < 0.05).

4 Discussion

Analyses of intestinal health parameters in this trial confirmed that inclusion of oregano oil into the drinking water did not influence the pH value in the broiler excreta. This data indicates that intestinal processes including microbial metabolism were not remarkably affected by supplements added to drinking water. Consistent with our findings, HONG et al. (2012) reported that addition to the diet of broilers of a mixture of essential oils (125 mg/kg consisting of essential oils derived from oregano, anis and citrus peel) had no effect on intestinal pH values of different intestinal segments, i.e., duodenum, jejunum and ileum. Other authors reported that pH of the caecal content of broilers also was not different in response to the addition of a specific blend of essential oil components to the diet (CERISUELO et al., 2014) or when different protein sources (vegetable versus animal protein) in broiler chicken diets were used (Hossain et al., 2013). CHANG and CHEN (2003) have reported that inclusion of probiotics in the diet reduced excreta pH in broilers, which may indicate effects on composition and activity of the intestinal microbiota. No such effect was observed with the specific probiotic which was administered with drinking water.

Different additives given into drinking water did not influence excreta dry matter content of broilers in our study, only a small, non-significant increase was observed for broilers in the oregano oil group in the finisher phase. Similarly, LEE et al. (2003) reported that, after supplementing the feed with cinnamaldehyde, the excreta dry matter content was slightly higher than that of other experimental groups. If these small differences could be amplified and corroborated in future studies, broilers would benefit from these additives in terms of drier litter and would help to prevent the occurrence of breast blisters, skin burns, bruising, and condemnations, and the production of ammonia in the broiler farms. Our study however did not show a marked effect on excreta dry atter content and thus, further studies are advisable to investigate potential beneficial effects on broiler health and the environment.

Villus height is an important indicator of intestinal activity, while crypt depth and villus height to crypt depth ratio provide valuable information about the cellular turnover at the villus surface (ASHARAF et al., 2020). Additives can have a positive effect on the development of beneficial bacteria and improvement in growth of the anatomical structure in the intestine (OLNOOD et al., 2015). In this study, the ileum villus height was higher in broilers in the oregano oil-probiotic and probiotic groups than in oregano oil and control groups. Intestinal villus height depends on the balance between proliferation, migration, and cell apoptosis. The villi play a vital role in nutritional absorption, an activity which is partly influenced by their size (FONSECA-GARCIA et al., 2017). It has been documented that a probiotic *E. Faecium* strain as a feed additive increased villus height in the ileum of broilers (SAMLI et al., 2007), which is in line with our results. Moreover, others have reported that an addition of a *Lactobacillus* species as probiotic in drinking water also increased villus height in the ileum of broilers (HIDAYAT et al., 2018).

In our study, crypt depth was greater in the ileum in broilers in the probiotic group than in the oregano oil-probiotic group. PELICANO et al. (2005) observed a greater crypt depth in the ileum in broilers in a probiotic group (probiotics based on *Bacillus subtilis*) than in other experimental groups. Crypts in the intestine are the source of epithelial cells for villi and crypt depth is strongly linked with epithelial cell turnover. Thus, shorter crypts imply a reduction in cellular turnover and improved intestinal health (MURUGESAN et al., 2015). In our study villus height to crypt depth ratio was higher in the ileum in broilers in the oregano oil-probiotic group than other experimental groups. As it has been described, higher villus height and deeper crypt depth leads to a higher villus to crypt depth ratio, which is an indicator of mature enterocytes at the villus tips of birds, a balanced enterocyte migration and sloughing, and efficient function for nutrient absorption for optimal growth (PAIVA et al., 2014). Deeper crypt depth and higher villus to crypt depth ratio in the intestine is an improvement indicator of its health by proving the positive influence of the additive on the digestive system (DRASKOVIC et al., 2020) and its overall stability. This, in turn, would also provide a better intestinal environment for the growth of beneficial bacteria.

5 Conclusions

Supplementation of drinking water with an oregano oil-probiotic additive had positive effects on the histomorphological parameters villus height and crypt depth and accordingly villus height to crypt depth ratio in broilers. However, oregano oil, a probiotic, and combination of both additives given into drinking water did not have any influence on pH and excreta dry matter content in broiler chickens indicating undisturbed digestion in all experimental groups. Future studies should be conducted applying varying concentrations of the additives supplied with drinking water.

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References

ASHARAF, S., H. ZANEB, S. MASOOD, S. YOUSAF, M.M. USMAN, H.F. REHMAN, A. SIKANDAR, M. SHAH and H. REHMAN (2020): Growth performance, hormonal dynamics and intestinal microarchitecture in broilers fed β-galacto-oligosaccharides during cyclic cold stress. J. Anim. Plant Sci. **30,** 288–297.

AVILA RAMOS, F., A. PRO MARTINEZ, E. SOSA MONTES, C. NARCISO GAYTAN, A.S. HERNANDEZ CAZAREZ, J. CIBRIAN TOVAR, J. GALLEGOS SANCHEZ and J.C. RODRIGUEZ CASTILLO (2017): Oregano oil use in broiler diet increases accumulation of carvacrol and thymol in breast meat. Acta Univ. **27,** 34–39.

AWAD, W., K. GHAREEB and J. BOHM (2008): Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing *Enterococcus faecium* and oligosaccharides. Int. J. Mol. Sci. **9**, 2205–2216.

BURT, S. (2004): Essential oils: Their antibacterial properties and potential applications in foods – A review. Int. J. Food Microbiol. **94,** 223–253.

CASTANON, J.I.R. (2007): History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. **86**, 2466–2471.

CERISUELO, A., C. MARIN, F. SANCHEZ-VIZCAINO, E.A. GOMEZ, J.M. DE LA FUENTE, R. DURAN and C. FERNANDEZ (2014): The impact of a specific blend of essential oil components and sodium butyrate in feed on growth performance and Salmonella counts in experimentally challenged broilers. Poult. Sci. **93,** 599-606.

CHANG, M.H. and T.C. CHEN (2003): Reduction of broiler house malodor by direct feeding of a Lactobacilli containing probiotic. Int. J. Poult. **2,** 313–317.

COLLETT, S.R. (2012): Nutrition and wet litter problems in poultry. Anim. Feed Sci. Technol. **173,** 65–75.

DHAMA, K., V. VERMA, P.M. SAWANT, R. TIWARI, R.K. VAID and R.S. CHAUHAN (2011): Applications of probiotics in poultry: Enhancing immunity and beneficial effects on production performances and health. A Review. J. Immunol. Immunopath. **13,** 1–19.

DRASKOVIC, V., Z. STANIMIROVIC, M. GLISIC, J. BOSNJAK-NEUMULLER, R. TEODOROVIC, V. TEODOROVIĆ and V. KUKOLJ (2020): The effects of a phytogenic additive on the histomorphometric characteristics of the intestines in weaned pigs with a subclinical natural infection with lawsonia intracellularis. Acta Vet. **70,** 81 – 91.

DUNCAN, D.B. (1955): Multiple range and multiple F-tests. Biometrics. **11,** 1–42.

FONSECA-GARCIA, I., F. ESCALERA-VALENTE, S. MATINEZ GONZALEZ, C.A. CARMONA-GASCA, D.A. GUTIEREZ-ARENAS and F. AVILA-RAMOS (2017): Effect of oregano oil dietary supplementation on production parameters, height of intestinal villi and the antioxidant capacity in the breast of broiler. Aust. J. Vet. Sci. **49,** 83–89.

GUO, F.C., H.F.J. SAVELKOUL, R.P. KWAKKEL, B.A. WILLIAMS and M.W.A. VERSTEGEN (2003): Immunoactive, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. World's Poult. Sci J. **59,** 427–440.

HIDAYAT, M.N., R. MALAKA, L. AGUSTINA and W. PAKIDING (2018): Effect of Lactobacillus sp. probiotics on intestinal histology, Escherichia coli in excreta and broiler performance. J. Indonesian Trop. Anim. Agric. **43,** 445–452.

HONG. J.C., T. STEINER. A. AUFY and T.F. LIEN (2012): Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. Livest. Sci. **144,** 253–262.

HOSSAIN. M.A., A.F. ISLAM and P.A. IJI (2013): Growth responses, excreta quality, nutrient digestibility, bone development and meat yield traits of broiler chickens fed vegetable or animal protein diets. S. Afr. J. Anim. Sci. **43,** 208–218.

HOSSAIN. M.M., M. BEGUM and I.H. KIM (2015): Effect of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on growth performance, nutrient digesti bility, meat quality, relative organ weight, microbial shedding and excreta noxious gas emission in broilers. Vet. Med. **60,** 77–86.

HUYGHEBAERT, G., R. DUCATELLE and F. VAN IMMERSEEL (2011): An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. **187,** 182–188.

IZADI, M., H.-J. ALERT and K.-H. SÜDEKUM (2025a): The effect of inclusion in drinking water, either singly or alternating, of oregano oil and a probiotic *Enterococcus faecium* strain on feed and water intake of growing broiler chickens. Züchtungsk. **97**, 123–135.

IZADI, M., H.-J. ALERT and K.-H. SÜDEKUM (2025b): The effect of inclusion in drinking water, either singly or alternating, of oregano oil and a probiotic *Enterococcus faecium* strain on performance of growing broiler chickens. Züchtungsk. **97**, 136–144.

KARIMI, A., F. YAN, C. COTO, J.H. PARK, Y. MIN, C. LU, J.A. GIDDEN, J.O. LAY JR and P.W. WALDROP (2010): Effects of level and source of oregano leaf in starter diets for broiler chicks. J. Appl. Poult. Res. **19,** 137–145.

KAWALILAK, L.T., A.M. ULMER FRANCO and G.M. FASENKO (2010): Impaired intestinal villi growth in broiler chicks with unhealed navels. Poult. Sci. **89,** 82–87.

KOGUT, M.H. (2013): The gut microbiota and host innate immunity: Regulators of host metabolism and metabolic diseases in poultry? J. Appl. Poult. Res. **22,** 637–646.

LEE, K.W., H. EVERTS, H.J. KAPPERT, M. FREHNER, R. LOSA and A.C. BEYNEN (2003): Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br. Poult. Sci. **44,** 450–457.

LEYVA-LOPEZ, N., E.P. GUTIERREZ-GRIJALVA, G. VAZQUEZ-OLIVO and J.B. HEREDIA (2017): Essential oils of oregano: Biological activity beyond their antimicrobial properties. Molecules. **22,** 989.

MURUGESAN, G.R., B. SYED, S. HALDAR and C. PENDER (2015): Phytogenic feed additives as an alternative to antibiotic growth promoters in broiler chickens. Front. Vet. Sci. **2,** 21.

NHUNG, N.T., N. CHANSIRIPORNCHAI and J.J. CARRIQUE-MAS (2017): Antimicrobial resistance in bacterial poultry pathogens: A Review. Front. Vet. Sci. **4,** 1–17.

OLNOOD, C.G., S.S.M. BESKI and P.A.IJI and M. CHOCT (2015): Delivery routes for probiotics: Effects on broiler performance, intestinal morphology and gut microflora. Anim Nutr. **1,** 192–202.

PAIVA, D., C. WALK and A. MCELROY (2014): Dietary calcium, phosphorus, and phytase effects on bird performance, intestinal morphology, mineral digestibility, and bone ash during a natural necrotic enteritis episode. Poult Sci. **93,** 2752–2762.

PELICANO, E.R.L., P.A. SOUZA, H.B.A. SOUZA, D.F. FIGUEIREDO, M.M. BOIAGO, S.R. CARVALHO and V.F. BORDON (2005): Intestinal mucosa development in broiler chickens fed natural growth promoters. Braz. J. Poult. Sci. **7,** 221–229.

PRAKATUR, I., M. MISKULIN, M. PAVIC, K. MARJANOVIC, V. BLAZICEVIC, I. ISKULIN and M. DOMACINOVIC (2019): Intestinal morphology in broiler chickens supplemented with propolis and bee pollen. Animals **9,** 301.

RIVENIA, S. and H. ASYROFI (2015): Comparison of antibiotic and probiotic as growth promoters using histological analysis on chicken intestine. Proc. Hokkaido Indonesian Stud. Assoc. Sci. Meet (HISAS), Hokkaido, Japan. **XII,** 2–7.

SAMLI, H.E., N. SENKOYLU, F. KOC, M. KANTER and A. AGMA (2007): Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Arch. Anim. Nutr. **61,** 42 – 49.

SEAL, B.S., H.S. LILLEHOJ, D.M. DONOVAN and C.G. GAY (2013): Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. Anim. Health Res. Rev. **14,** 78–87.

SOHAIL, M.U., M.E. HUME, J.A. BYRD, D.J. NISBET, A. IJAZ, A. SOHAIL, M.Z. SHABBIR and H. REHMAN (2012): Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult Sci. **91,** 2235–2240.

SUGIHARTO, S. (2016): Role of nutraceuticals in gut health and growth performance of poultry. J. Saudi Soc. Agric. Sci. **15,** 99–111.

SVIHUS, B. (2014): Function of the digestive system. J. Appl. Poult. Res. **23,** 306–314.

TURK, D.E. (1982): The anatomy of the avian digestive tract as related to feed utilization. Poult. Sci. **61,** 1225–1244.

VDLUFA [Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten]. (2012): Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA Methodenbuch), Bd. III. Die Chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

WENK, C. (2003): Herbs and botanicals as feed additives in monogastric animals. Asian-Australas. J. Anim. Sci. **16,** 282–289.

WPSA, World's Poultry Science Association (1984): Working Group No. 2 – Nutrition: the prediction of apparent metabolizable energy values for poultry in compound feeds. World's Poult. Sci. J. **40,** 181–182.

YADAV, S. and R. JHA (2019): Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J. Anim. Sci. Biotechnol. **10,** 1–11.

YIN, D., E. DU, J. YUAN, J. GAO, Y. WANG, S.E. AGGREY and Y. GUO (2017): Supplemental thymol and carvacrol increases ileum *Lactobacillus* population and reduces effect of necrotic enteritis caused by *Clostridium perfringens* in chickens. Sci. Rep. **7,** 7334.

YITBAREK, M.B. (2015): Phytogenics as feed additives in poultry production: A Review. International J. Ext. Res. **3,** 49–60.

YU, L.C., J.T., WANG, S.C. WIE and Y.H. NI (2012): Host-microbial interactions and regulation of intestinal epithelial barrier function: From physiology to pathology. World J Gastrointest. Pathophysiol. **3,** 27–43.

CHAPTER 6

Final considerations

6.1. Gut health in broilers

Gut health in broilers is a key issue not only for animal health but also for overall performance as birds with better gut health have an improved digestion and absorption of nutrients. Antibiotic additives may or have at least been suspected to negatively impact on the healthiness of people and, therefore, it is advisable to search for substitutes for antibiotics.

6.1.1. Inclusion of additives in drinking water and excreta characteristics of broilers

Analysis of intestinal health parameters in this study confirmed that the inclusion of oregano oil in drinking water did not change the pH value in the faeces of broilers. Our findings is to some extent in agreement with the results of (Hong *et al*., 2012) who reported that by adding EO (125 ppm including EO derived from oregano, anis and citrus peel), there was no observed change in intestinal pH values of the different intestinal segments; duodenum, jejunum and ileum in broilers. In addition, based on research, it was reported that the pH of the cecal content was not dissimilar by the addition of specific blend of EO components in the diet of broilers (Cerisuelo *et al*., 2014). Hossain *et al*. (2013) reported that excreta pH was not changed by different protein sources (vegetable or animal protein) in broiler chicken diets and pH value was an average of 7.3, however, in our study, it was used other natural additives. Han *et al*. (1999) found that fecal pH was not altered by feeding *aspergillus oryzae* culture as probiotic to the diet of laying hens, which agrees with our result. There are not too many published data concerning excreta pH in broilers available.

In our study, no influence of feed additives supplied with drinking water on excreta DM concentration of broilers was observed. Similarly, when cinnamaldehyde was added to the diet, Lee *et al*. (2003) detected that the excreta DM concentration was only very slightly higher than that of other experimental groups such that no proof can be given that specific feed additives supplied with either drinking water or the diet have an impact on excreta DM concentration.

6.1.2. Inclusion of additives in drinking water and histomorphological characteristics in broilers

The small intestine is an important digestive organ involved in nutrient absorption and growth, and the development of this organ is important for the health and performance of broilers (Kawalilak *et al.* 2010).

Villus height is an important indicator of intestinal activity, while crypt depth and villus height to crypt depth ratio provide valuable information about the cellular turnover at the villus surface (Ashraf *et al*., 2020). Small intestinal villus is the protrusion of the lamina propria into the intestinal lumen that increases the digestive and absorptive surface area in the intestine (Yamauchi, 2002). Intestinal villus height depends on the balance between proliferation, migration, and cell apoptosis. The villi play an important role in nutrient absorption, an activity which is partly dependent on its size (Fonseca-García *et al*., 2017). The activity of intestinal mucosa can be affected by the composition of the feed and the intestinal microbiota (Bucław, 2016). Mucosa status and their microscopic structure can be a reliable indicator of the response of the intestinal tract to active substances in feeds (Jamroz *et al*., 2006).

It has been noted that probiotics strengthen the function of the intestinal mucosa in the GIT against harmful substances (Hidayat *et al*., 2018) by mechanisms such as bacterial antagonisms, competitive exclusion and immune stimulation which improve microbial balance in the GIT (Awad *et al*., 2008). It has also been reported that thymol and carvacrol limit bacterial growth and modulate the pathogenicity of the bacteria in the gut (Yin *et al*., 2017).

In this study, the ileum villus height was higher in the oregano oil/probiotic and probiotic groups than CON group, and a trend was observed for an increase in villus height in the ileum of the oregano oil group. Hussein *et al*. (2020) reported that probiotic combined with PFA modulated gut health by a reduction in lesion scores in *Clostridium perfringens* challenged birds in the intestine of broilers. The authors concluded that simultaneous application of a probiotic and PFA advantageously affects gut health in broilers. Svihus (2014) reported that increased villus height may be induced by an increased need for digestive capacity in the intestinal segments. In our study, only a tendency was observed to higher villus height in the ileum in the oregano oil group, whereas Dehghani *et al*. (2018) reported an increase in villus height of the ileum in quail supplemented with different levels of savory and thyme essential oils in the diet. A PFA commodity (Digestarom®Poultry) at 150 mg/kg increased villus height compared to the CON group in the ileum of broiler chickens (Murugesan *et al*., 2015).

Pluske *et al*. (1996) have assumed that maximum digestion and absorption are positively correlated with higher villi, most likely because longer villi increase the absorptive surface of the intestine (Markovic *et al*., 2009). The structure of the intestinal mucosa can show information on gut health as stressors that are present in the digesta result in relatively quick changes in the intestinal mucosa because of the close proximity of the mucosal surface and the intestinal content (Xu *et al*., 2003). Yamauchi (2007) noted that despite several studies reported on intestinal histomorphological features, the link between these features and intestinal function and/or nutritional value of the feed and, eventually, productivity of animals has not been precisely studied.

The intestinal tract, however, makes up only 5% of the total BW, but it uses 30% of the whole body's oxygen consumption and nutrient turnover at maintenance metabolism (Perry, 2006). Any additional tissue turnover will lead to an increase in energy and nutrient requirements for maintenance and result in lower efficiency of the animal. Shortening of villi and deeper crypts lead to poor nutrient absorption, increased secretion in the GIT and reduced performance (Montagne *et al*., 2003; Xu *et al*., 2003).

The crypt can be regarded as the villus factory and a large crypt indicates rapid tissue turnover and a high demand for new tissue. Pelicano *et al*. (2005) observed deeper ileum crypts with the use of probiotic in the diet of broilers, which allow for an improved villus regeneration (Bogucka *et al*., 2019). *Enterococcus faecium* as a feed additive increased villus height in the ileum of broilers (Samli *et al*., 2007), which is corroborated by our results. Moreover, an increase in villus height in the ileum was also observed by the addition of probiotic *Lactobacillus sp.* in drinking water of broilers (Hidayat *et al*., 2018). Bogucka *et al*. (2019) reported that crypt depth was increased significantly with probiotic supplementation in the diet of broiler chickens which allows for larger villus regeneration. Awad *et al*. (2009) reported that dietary supplementation with probiotic increased villus height to crypt depth ratio and tended to increase villus height in the ileum of broilers which is consistent with our result that probiotic group compared to CON and oregano oil groups increased villus height to crypt depth ratio in the intestine of broilers. On the contrary, Shams shargh *et al*. (2012) reported that villus height, crypt depth, and villus height to crypt depth ratio in the ileum were not affected by the treatments including two plant extracts (garlic and thyme), and probiotic as additives into drinking water of broilers. In our study, crypt depth was greater in probiotic group than in other experimental groups. Previously, Pelicano *et al*. (2005) reported a greater crypt depth in ileum of birds supplemented with *Bacillus subtilis*-based probiotic than in other experimental groups.

Crypt width was not altered by the inclusion of different additives. A more detailed discussion of our findings regarding application of probiotic and oregano oil with drinking water and their effects on histomorphological characteristics would require a greater number of studies that investigated the effects of probiotic and oregano oil in drinking water of broilers on villus height, crypt depth, villus height to crypt depth ratio, and crypt width.

6.2. Effects of additives in drinking water on broiler performance

One hypothesis of our study was that supplementing the drinking water with probiotic, oregano oil, and oregano oil/probiotic would stimulate growth performance in broiler chickens. Numerous studies have been conducted on the supplementation of probiotics in the diet with varying and inconsistent effects on broiler performance. Only very few studies have focused on inclusion of probiotics into drinking water and their effects on feed intake and performance. Hidayat *et al*. (2018) observed that the inclusion of *Lactobacillus* sp. in drinking water, did not affect feed intake which is consistent with our findings. On the contrary, incorporation of a multispecies and a chicken-specific probiotic preparation in fluid form via drinking water improved broiler performance (Timmerman *et al*., 2006). Moreover, it has been stated that the application of multi-strain probiotic in the drinking water may improve BW and WG in broilers (Steiner, 2009).

REFERENCES FOR CHAPTER 6

Ashraf, S., Zaneb, H., Masood, S., Yousaf, S., Usman, M.M., Rehman, H.F., Sikandar, A., Shah, M., & Rehman, H. (2020). Growth performance, hormonal dynamics and intestinal microarchitecture in broilers fed β-galacto-oligosaccharides during cyclic cold stress. *JAPS, Journal of Animal and Plant Sciences*, 30, 288-297.

Awad, W. A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, 88, 49-56.

Bogucka, J., Ribeiro, D. M., Bogusławska-Tryk, M., Dankowiakowska, A., da Costa, R. P. R., & Bednarczyk, M. (2019). Microstructure of the small intestine in broiler chickens fed a diet with probiotic or synbiotic supplementation. *Journal of Animal Physiology and Animal Nutrition*, 103, 1785-1791.

Bucław, M. (2016). The use of inulin in poultry feeding: a review. *Journal of Animal Physiology and Animal Nutrition*, 100, 1015-1022.

Cerisuelo, A., Marín, C., Sánchez-Vizcaino, F., Gómez, E. A., De La Fuente, J. M., Durán, R., & Fernández, C. (2014). The impact of a specific blend of essential oil components and sodium butyrate in feed on growth performance and Salmonella counts in experimentally challenged broilers. *Poultry Science*, 93, 599-606.

Dehghani, N., Afsharmanesh, M., Salarmoini, M., Ebrahimnejad, H., & Bitaraf, A. (2018). Effect of pennyroyal, savory and thyme essential oils on Japanese quail physiology. *Heliyon*, 4. e00881.

Fonseca-García, I., Escalera-Valente, F., Martínez-González, S., Carmona-Gasca, C. A., Gutiérrez-Arenas, D. A., & Ávila-Ramos, F. (2017). Effect of oregano oil dietary supplementation on production parameters, height of intestinal villi and the antioxidant capacity in the breast of broiler. *Austral Journal of Veterinary Sciences*, 49, 83-89.

Han, S. W., Lee, K. W., Lee, B. D., & Sung, C. G. (1999). Effect of feeding Aspergillus oryzae culture on fecal microflora, egg qualities, and nutrient metabolizabilities in laying hens. *Asian-Australasian Journal of Animal Sciences*, 12, 417-421.

Hidayat, M. N., Malaka, R., Agustina, L., & Pakiding, W. (2018). Effect of *Lactobacillus* sp. probiotics on intestinal histology, Escherichia coli in excreta and broiler performance. *Journal of the lndonesian Tropical Animal Agriculture*, 43, 445-452.

Hong, J. C., Steiner, T., Aufy, A., & Lien, T. F. (2012). Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. *Livestock Science*, *144*, 253-262.

Hossain, M. A., Islam, A. F., & Iji, P. A. (2013). Growth responses, excreta quality, nutrient digestibility, bone development and meat yield traits of broiler chickens fed vegetable or animal protein diets. *South African Journal of Animal Science*, 43, 208-218.

Hussein, E. O., Ahmed, S. H., Abudabos, A. M., Suliman, G. M., Abd El-Hack, M. E., Swelum, A. A., & N. Alowaimer, A. (2020). Ameliorative effects of antibiotic-, probiotic-and phytobiotic-supplemented diets on the performance, intestinal health, carcass traits, and meat quality of Clostridium perfringens-infected broilers. *Animals*, 10, 669.

Jamroz, D., Wertelecki, T., Houszka, M., & Kamel, C. (2006). Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *Journal of Animal Physiology and Animal Nutrition*, 90, 255-268.

Kawalilak, L. T., Franco, A. U., & Fasenko, G. M. (2010). Impaired intestinal villi growth in broiler chicks with unhealed navels. *Poultry Science*, 89, 82-87.

Lee, K. W., Everts, H., Kappert, H. J., Frehner, M., Losa, R., & Beynen, A. C. (2003). Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. *British Poultry Science*, 44, 450-457.

Marković, R., Šefer, D., Krstić, M., & Petrujkić, B. (2009). Effect of different growth promoters on broiler performance and gut morphology. *Archivos de Medicina Veterinaria*, *41*, 163-169.

Montagne, L., Pluske, J. R., & Hampson, D. J. (2003). A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology*, *108*, 95-117.

Murugesan, G.R., Syed, B., Haldar, S., & Pender, C. (2015). Phytogenic feed additives as an alternative to antibiotic growth promoters in broiler chickens. *Frontiers in Veterinary. Science* 2, 21.

Pelicano, E. R. L., Souza, P. D., Souza, H. D., Figueiredo, D. F., Boiago, M. M., Carvalho, S. R., & Bordon, V. F. (2005). Intestinal mucosa development in broiler chickens fed natural growth promoters. *Brazilian Journal of Poultry Science*, 7, 221-229.

Perry, G.C. (Ed.) (2006). *Avian gut function in health and disease*. Poultry Science Symposium Series, Vol. 28. CABI Publishing, Wallingford, UK.

Pluske, J. R., Tompson, M. J., Atwood, C. S., Bird, P. H., Wiliams, I. H., & Hartmann, P. E. (1996). Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. *British Journal of Nutrition*, 76, 409–422.

Samli, H. E., Senkoylu, N., Koc, F., Kanter, M., & Agma, A. (2007). Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. *Archives of animal nutrition*, 61, 42-49.

Shams Shargh, M., Dastar, B., Zerehdaran, S., Khomeiri, M., & Moradi, A. (2012). Effects of using plant extracts and a probiotic on performance, intestinal morphology, and microflora population in broilers. *Journal of Applied Poultry Research*, 21, 201-208.

Steiner, T. (2009). Probiotics in poultry and pig nutrition: Basics and benefits. *Feed and Nutrition*, 55-58.

Svihus, B. (2014). Function of the digestive system. *Journal of Applied Poultry Research*, 23, 306-314.

Timmerman, H. M., Veldman, A., Van den Elsen, E., Rombouts, F. M., & Beynen, A. C. (2006). Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. *Poultry science*, 85, 1383-1388.

Xu, Z. R., Hu, C. H., Xia, M. S., Zhan, X. A., & Wang, M. Q. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science*, 82, 1030-1036.

Yamauchi, K. E. (2007). Review of a histological intestinal approach to assessing the intestinal function in chickens and pigs. *Animal Science Journal*, 78, 356-370.

Yamauchi, K. E. (2002). Review on chicken intestinal villus histological alterations related with intestinal function. *The Journal of Poultry Science*, 39, 229-242.

Yin, D., Du, E., Yuan, J., Gao, J., Wang, Y., Aggrey, S. E., & Guo, Y. (2017). Supplemental thymol and carvacrol increases ileum *Lactobacillus* population and reduces effect of necrotic enteritis caused by *Clostridium perfringes* in chickens. *Scientific Reports*, 7, 7334.

7. Conclusions

This study was designed to examine the inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, on growing broiler chickens. Three major hypotheses were tested:

1. The inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would not negatively affect feed and water intake of broiler chickens during a 42-day growth period.
2. The inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would improve growth performance of broiler chickens during a 42-day growth period.
3. The inclusion of oregano oil and a probiotic *Enterococcus faecium* strain in drinking water, either singly or alternating, would positively impact on gut health characteristics of growing broiler chickens.

From the results of the experiment, the following conclusions can be made:

1. Inclusion of various additives administered through drinking water had no significant effects on daily water intake in broiler chicken. Moreover, water to feed intake ratio as a tool for measurement of adequate water intake in broilers was not significantly influenced by additives. Hence the administration with drinking water may be a viable way to supply oregano oil or a probiotic *Enterococcus faecium* strain, or both, to diets of young, rapidly growing chicken without compromising their acceptance to consume water. Further studies are needed in order to elucidate the effects of different levels of these feed additives on water and feed intake in broilers.

2. Inclusion of various additives administered through drinking water had no effects on growth performance, feed conversion ratio and on liver and abdominal fat pad weights. Although no beneficial responses of broilers were observed to the supply of additives with drinking water, the absence of adverse effects, shows, for the investigated types of additives, the general applicability of the route of administration, i.e., supply in drinking water and not in feed. Therefore, it appears justified to conduct further studies in order to elucidate the effects of different levels of these feed additives on broiler performance when supplied in drinking water.

3. Supplementation of drinking water with an oregano oil-probiotic additive had positive effects on the histomorphological parameters villus height and crypt depth and accordingly villus height to crypt depth ratio in broilers. However, oregano oil, a probiotic, and combination of both additives given into drinking water did not have any influence on pH and excreta dry matter content in broiler chickens indicating undisturbed digestion in all experimental groups.

Table A1: Effects of supplements added to drinking water on average daily water intake (g/bird) of broilers during a 42-day growth period

Age (days)	*Experimental groups* Control	Probiotic	Oregano oil	Oregano oil/Probiotic	Pooled SEM	*Probability*
1	16.5^{bc*} ±2.58	17.9^{b} ±2.24	15.9^{c} ±1.90	20.23^{a} ±1.45	0.415	**<0.001**
2	33.8^{a} ±2.09	35.7^{a} ±3.67	30.6^{b} ±3.19	31.02^{b} ±2.81	0.565	**≤0.001**
3	45.7$^{(a)**}$±4.38	45.2$^{(a)}$±4.37	42.8$^{(ab)}$±4.19	40.7$^{(b)}$±4.41	0.732	0.054
4	52.5±4.24	51.4±4.06	52.4±4.11	54.29±5.60	0.710	0.566
5	62.4±4.86	61.4±2.96	63.2±3.49	63.42±4.74	0.635	0.692
6	72.6±4.79	71.3±3.00	72.0±2.86	72.4±4.45	0.593	0.884
7	93.0±3.34	92.9±4.86	91.2±5.35	93.6±2.49	0.649	0.629
8	75.4±6.62	69.9±4.51	74.0±4.83	73.98±3.36	0.822	0.100
9	93.9^{a}±4.20	90.8^{ab}±6.82	88.1^{bc}±7.65	85.1^{c}±4.30	1.04	**0.014**
10	102±4.82	102±5.21	99.0±7.23	104±6.12	0.942	0.328
11	98±11.06	98.6±9.83	93.6±8.84	101±7.71	1.50	0.367
12	105±6.96	103±5.55	98.7±7.85	106±6.90	1.12	0.144
13	122±7.60	121±6.92	116±9.01	124±10.9	1.40	0.260
14	145±11.45	147±7.48	139±9.31	146±11.1	1.59	0.282
15	105^{ab}±5.85	110^{a}±8.63	93.7^{c}±12.0	98.3^{bc}±6.73	1.63	**≤0.001**
16	139±7.05	140±9.95	134±9.20	134±7.35	1.36	0.248
17	147±10.2	146±18.2	141±7.67	149±15.2	2.09	0.663
18	161^{a}±11.3	152^{ab}±9.00	144^{b}±13.8	151^{ab}±8.29	1.90	**<0.05**
19	171^{bc}±15.0	175^{ab}±13.6	161^{c}±18.9	186^{a}±9.79	2.65	**<0.01**
20	176±16.0	173±13.8	169±22.2	180±7.32	2.48	0.460
21	190±18.1	189±15.9	187±13.4	196±10.4	2.30	0.541
22	178±19.5	167±15.6	169±19.3	175±14.5	2.74	0.419
23	211±16.2	201±16.8	196±14.2	199±15.9	2.58	0.161
24	210±20.2	211±19.5	207±16.9	213±14.0	2.73	0.894
25	233$^{(ab)}$±13.7	235$^{(a)}$±16.3	220$^{(b)}$±12.7	232$^{(ab)}$±12.5	2.30	0.092
26	221$^{(a)}$±12.3	221$^{(a)}$±13.3	206$^{(b)}$±14.2	217$^{(ab)}$±13.2	2.24	0.053

27	235±8.04	234±17.5	223±13.6	227±9.98	2.11	0.112
28	275^{ab}±10.61	284^{a}±12.1	271^{ab}±14.7	266^{b}±18.4	2.42	**<0.05**
29	238±15.3	250±17.4	243±13.2	248±16.3	2.49	0.326
30	242^{a}±19.1	233^{ab}±12.6	221^{b}±19.8	227^{ab}±12.1	2.77	**<0.05**
31	279^{ab}±10.1	284^{a}±13.3	270^{b}±17.9	286^{a}±9.07	2.22	**<0.05**
32	294^{a}±16.7	291^{a}±12.4	268^{b}±28.4	291^{a}±14.2	3.31	**<0.05**
33	270±16.2	270±8.92	259±18.4	276±14.8	2.48	0.110
34	300±16.9	303±10.3	291±21.3	296±18.4	2.71	0.436
35	343±17.8	349±10.0	339±22.9	353±26.3	3.19	0.418
36	256^{b}±11.8	255^{b}±14.7	259^{b}±22.4	287^{a}±21.3	3.47	**≤0.01**
37	306±19.3	300±17.9	293±15.0	285±25.7	3.26	0.134
38	251$^{(ab)}$±17.9	249$^{(b)}$±15.1	250$^{(ab)}$±25.1	270$^{(a)}$±26.2	3.57	0.097
39	323±28.1	303±12.2	305±24.8	303±28.4	3.93	0.191
40	364$^{(a)}$±20.0	346$^{(ab)}$±15.7	334$^{(b)}$±26.3	349$^{(ab)}$±28.5	3.92	0.053
41	332±24.9	315±18.9	313±25.4	312±26.8	3.90	0.229
42	390±32.3	373±27.1	363±28.1	365±20.8	4.51	0.117
1-42 d	189±6.15	187±7.16	181±9.83	188±8.03	1.30	0.115

Values are presented as mean±SD
$^{a, b, c}$ Means in rows showing different superscripts are different at P<0.05
Abbreviations: SEM, standard error of means.

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